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(57) Abstract

A method for killing pests (e.g. insects) comprising administering material from *Xenorhabdus* species (e.g. *X. nematophilus*) such as cells or supernatants orally to the pests, either alone or in conjunction with *Bacillus thuringiensis* or pesticidal materials derived therefrom. Also disclosed is an isolated pesticidal agent (and compositions comprising the same) characterised in that it is obtainable from cultures of *X. nematophilus* or mutants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55 °C, is proteinaceous, acts synergistically with *B. thuringiensis* cells as an oral pesticide and is substantially resistant to proteolysis by trypsin and proteinase K. DNA encoding pesticidal activity is also disclosed.

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PESTICIDAL AGENTS

The present invention relates to materials, agents and compositions having pesticidal activity which derive from bacteria, and more particularly from *Xenorhabdus* species. The invention further relates to organisms and methods employing such compounds and compositions.

There is an ongoing requirement for materials, agents, compositions and organisms having pesticidal activity, for instance for use in crop protection or insect-mediated disease control. Novel materials are required to overcome the problem of resistence to existing pesticides. Ideally such materials are cheap to produce, stable, have a high toxicity (either when used alone or in combination) and are effective when taken orally by the pest target. Thus any invention which provided materials, agents, compositions or organisms in which any of these properties was enhanced would represent a step forward in the art.

Xenorhabdus spp. in nature are frequently symbiotically associated with a nematode host, and it is known that this association may be used to control pest activity. For instance, it is known that certain *Xenorhabdus spp.* alone are capable of killing an insect host when injected into the host's hemocoel.

In addition, one extracellular insecticidal toxin from *Photorhabdus luminescens* has been isolated (this species was recently removed from the genus *Xenorhabdus*, and is closely related to the species therein). This toxin is not effective when ingested, but is highly toxic when injected into certain insect larvae (see Parasites and Pathogens of Insects Vol.2, Eds. Beckage, N. E. et al., Academic Press 1993).

Also known are certain low-molecular weight heterocyclic compounds from *P.luminescens* and *X.nematophilus* which have antibiotic properties when applied intravenously or topically (see Rhodes, S.H. et al., PCT WO 84/01775).

Unfortunately none of these prior art materials have the ideal pesticide characteristics discussed above, and in particular, they do not have toxic activity when administered orally.

The present invention provides pesticidal agents and compositions from *Xenorhabdus* species, organisms which produce such compounds and compositions, and methods which employ these agents, compositions and organisms, that alleviate some of the problems with the prior art.

According to one aspect of the present invention there is disclosed a method of killing or controlling insect pests comprising administering cells from *Xenorhabdus* species or pesticidal materials derived or obtainable therefrom, orally to the pests.

A PCT application of CSIRO published as WO 95/00647 discloses an apparently toxic protein from *Xenorhabdus nematophilus*; however no details of the protein's toxicity are given, and certainly there is no disclosure of its use as an oral insecticide.

Thus the invention provides an insecticidal composition adapted for oral administration to an insect, which composition comprises a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.

The composition may in fact comprise cells of *Xenorhabdus* or alternatively supernatant taken from cultures of cells of *Xenorhabdus* species. However, the composition

preferably comprises toxins isolable from *Xenorhabdus* as illustrated hereinafter. Toxic activity has been associated with material encoded by the nucleotide sequence of Figure 2. Thus, the composition suitably comprises a pesticidal material which is encoded by all or part of the nucleotide sequence of Figure 2. Pesticidal fragments as well as variants or derivatives of such toxins may also be employed.

The sequence of Figure 2 is of the order of 40kb in length. It is believed that this sequence may encode more than one protein, each of which may regulate or be insecticidal either alone or when presented together. It is a matter of routine to determine which parts are necessary or sufficient for insecticidal activity.

As used herein the term ``variant'' refers to toxins which have modified amino acid sequence but which share similar activity. Certain amino acids may be replaced with different amino acids without altering the nature of the activity in a significant way. The replacement may be by way of ``conservative substitution'' where an amino acid is replaced with an amino acid of broadly similar properties, or there may be some non-conservative substitutions. In general however, the variants will be at least 60% homologous to the native toxin, suitably at least 70% homologous and more preferably at least 90% homologous.

The term ``derivative'' relates to toxins which have been modified for example by chemical or biological methods.

These toxins are novel, and they and the nucleic acids which encode them form a further aspect of the invention.

A preferred *Xenorhabdus* species is the bacteria *X.nematophilus*. Particular strains of *X.nematophilus* which are useful in the context of the invention are

ATTC 19061 strain, available from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (NCIMB). In addition, suitable strains include two novel strains of *Xenorhabdus* which were deposited at the NCIMB on 10 July 1997 and were designated with repository numbers NCIMB 40886 and NCIMB 40887. These latter strains form a further aspect of the invention.

All strains have common characteristics as set out in the following Table 1.

Table 1

	Strains		
Characteristics	ATCC 19061	NCIMB 40887	NCIMB 40886
Gram strain	negative	negative	negative
Shape/size	rods up to 4μm long	rods up to 4μm long	rods up to 4μm long
Motile	Yes	Yes	Yes
Bioluminescent	No	No	No
Colour on NBTA*	blue	blue	blue
insecticidal on ingestion by insects	yes	yes	yes
Production of Antibiotics	yes	yes	yes
Resistant to ampicillin (50μg/ml)	yes	yes	yes
colony morphology/ colour	circular convex cream	circular convex cream	circular convex cream

*NBTA (Oxoid nutrient agar containing 0.0025% bromothymol blue and 0.004% tetrazolium chloride)

Preferably the pest target is an insect, and more preferably it is of the order Lepidoptera, particularly

Pieris brassicae, *Pieris rapae*, or *Plutella xylostella* or the order *Diptera*, particularly *Culex quinquefaciatus*.

In a preferred embodiment of the invention, cells from *Xenorhabdus* species or agents derived therefrom are used in conjunction with *Bacillus thuringiensis* as an oral pesticide.

In further embodiments, rather than using *Bacillus thuringiensis* itself, pesticidal materials obtainable from *B.thuringiensis* (e.g. delta endotoxins or other isolates) are used in conjunction with *Xenorhabdus* species.

The term 'obtainable from' is intended to embrace not only materials which have been isolated directly from the bacterium in question, but also those which have been subsequently cloned into and produced by other organisms.

Thus the unexpected discovery that bacteria of the genus *Xenorhabdus*(and materials derived therefrom) have pesticidal activity when ingested, and that such bacteria and materials can be used advantageously in conjunction with *B.thuringiensis* (and toxins or materials derived therefrom), forms the basis of a further aspect of the present invention. The pesticidal activity of *B.thuringiensis* isolates alone have been well documented. However, synergistic pesticidal activity between such isolates and bacteria of the *Xenorhabdus* species (or materials derived therefrom) has not previously been demonstrated.

In still further embodiments of the invention, culture supernatant taken from cultures of *Xenorhabdus* species, particularly *X. nematophilus*, is used in place of cells from *Xenorhabdus* species in the methods above.

All of these methods can be employed, inter alia, in pest control.

The invention also makes available pesticidal compositions comprising cells from *Xenorhabdus* species, preferably *X.nematophilus*, in combination with *B. thuringiensis*. As with the methods above, a pesticidal toxin from *B.thuringiensis* (preferably a delta endotoxin) may be used as an alternative to *B.thuringiensis* in the compositions of the present invention

Likewise, culture supernatant taken from cultures of *Xenorhabdus* species, preferably, *X.nematophilus* may be used in place of cells from *Xenorhabdus* species.

Such compositions can be employed, *inter alia*, for crop protection eg. by spraying crops, or for livestock protection. In addition, compositions of the invention may be used in vector control.

The invention further encompasses novel pesticidal agents which can be isolated from *Xenorhabdus spp*. Techniques for isolating such agents would be understood by the skilled person.

In particular, such techniques include the separation and identification of toxin proteins either at the protein level or at the DNA level.

The applicants have cloned and partially sequenced a region of DNA from *Xenorhabdus* NCIMB 40887 which region codes for insecticidal activity and this is shown as Figure 2 (SEQ ID NO. 1) hereinafter. Thus in a preferred embodiment the invention also provides a toxin which is encoded by DNA of SEQ ID No. 1 or a variant or fragment thereof.

The invention also provides a recombinant DNA which encodes such a toxin. The recombinant DNA of the invention may comprise the sequence of Figure 2 or a variant or fragment thereof. Other DNA sequences may encode similar proteins as a result of the degeneracy of the genetic code. All such sequences are encompassed by the invention.

The sequence provided herein is sufficient to allow probes to be produced which can be used to identify and subsequently to extract DNA of toxin genes. This DNA may then be cloned into vectors and host cells as is understood in the art.

DNA which comprises or hybridises with the sequence of Figure 2 under stringent conditions forms a further aspect of the invention.

The expression ``hybridises with'' means that the nucleotide sequence will anneal to all or part of the sequence of Figure 2 under stringent hybridisation conditions, for example those illustrated in ``Molecular Cloning'', A Laboratory Manual'' by Sambrook, Fritsch and Maniatis, Cold Spring Habor Laboratory Press, Cold Spring Harbor, N.Y.

The length of the sequence used in any particular analytical technique will depend upon the nature of the technique, the degree of complementarity of the sequence, the nature of the sequence and particularly the GC content of the probe or primer and the particular hybridisation conditions employed. Under high stringency, only sequences which are completely complementary will bind but under low stringency conditions, sequences which are 60% homologous to the target sequence, more suitably 80% homologous, will bind. Both high and low stringency conditions are encompassed by the term ``stringent conditions'' used herein.

Suitable fragments of the DNA of Figure 2, i.e. those which encode pesticidal agents may be identified using standard techniques. For example, transposon mutagenesis techniques may be used, for example as described by H.S. Siefert et al., Proc. Natl. Acad. Sci. USA, (1986) 83, 735-739. Vectors such as the cosmid cHRIMI, can be mutated using a variety of transposons and then screened for loss of insectidal activity. In this way regions of DNA encoding proteins responsible for toxic activity can be identified.

For example, the mini-transposon mTn3(HIS3) can be introduced into a toxic *Xenorhabdus* clone such as cHRIM1, hereinafter referred to as `clone 1', by electroporating cHRIM1 DNA into *E.coli* RDP146(pLB101) and mating this strain with *E.coli* RDP146(pOX38), followed by *E. coli* NS2114Sm. The final strain will contain cHRIM1DNA with a single insertion of the transposon mTn3(HIS3). These colonies can be cultured and tested for insecticidal activity as described in Example 8 hereinafter. Restriction mapping or DNA sequencing can be used to identify the insertion point of mTn3(HIS3) and hence the regions of DNA involved in toxicity. Similar approached can be used with other transposons such as Tn5 and mTn5.

Site directed mutagenesis of cHRIM1 as outlined in ``Molecular Cloning, A Laboratory Manual'' by Maniatis, Fritsch and Sambrook, (1982) Cold Spring Harbor, can also be used to test the importance of specific regions of DNA for toxic activity.

Alternatively, subcloning techniques can be used to identify regions of the cloned DNA which code for insecticidal activity. In this method, specific smaller fragments of the DNA are subcloned and the activity determined. To do this, cosmid DNA can be cut with a suitable restriction enzyme and ligated into a compatible

restriction site on a plasmid vector, such as pUC19. The ligation mix can be transformed into *E. coli* and transformed clones selected using a selection marker such as antibiotic resistance, which is coded for on the plasmid vector. Details of these techniques are described for example in Maniatis et al, supra, (see p390-391) and Methods in Molecular Biology, by L.G. Davies, M.D. Dibner and J.F. Battey, Elsevier, (see p222-224).

Individual colonies containing specific cloned fragments can be cultured and tested for activity as described in Example 8 hereinafter. Subclones with insecticidal activity can be further truncated using the same methodology to further identify regions of the DNA coding for activity.

The invention also discloses an isolated pesticidal agent characterised in that the agent is obtainable from cultures of *X. nematophilus* or variants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55°C, is proteinaceous, acts synergistically with *B.thuringiensis* cells as an oral pesticide and is substantially resistant to proteolysis by trypsin and proteinase K.

By 'substantially heat stable to 55°C' is meant that the agent retains some pesticidal activity when tested after heating the agent in suspension to 55°C for 10 minutes, and preferably retains at least 50% of the untreated activity.

By 'substantially resistant to proteolysis' is meant that the agent retains some pesticidal activity when exposed to proteases at 30°C for 2 hours and preferably retains at least 50% of the untreated activity.

By 'acts synergistically' is meant that the activity of the combination of components is greater than one might expect from the use of the components individually. For example, when used in conjunction with *B.thuringiensis* cells as an oral pesticide, the concentration of B. thuringiensis cellular material necessary to give 50% mortality in a P.brassicae when used alone is reduced by at least 80% when it is used in combination the agent at a concentration sufficient to give 25% mortality when the agent is used alone.

It has been found that the activity of the material is retained by 30 kDa cut-off filters but is only partly retained by 100 kDa filters.

Preferably the agent is still further characterised in that the pesticidal activity is lost through treatment at 25°C with sodium dodecyl sulphate (SDS - 0.1% 60 mins) and acetone (50%, 60 mins).

Clearly the characterising properties of the isolated agent described above can be utilised to purify it from, or enrich its concentration in, *Xenorhabdus* species cells and culture medium supernatants. Methods of purifying proteins from heterogenous mixtures are well known in the art (eg. ammonium sulphate precipitation, proteolysis, ultrafiltration with known molecular weight cut-off filters, ion-exchange chromatography, gel filtration, etc.). The oral pesticidal activity provides a convenient method of assaying the level of agent after each stage, or in each sample of eluent. Such methodology does not require inventive endeavour by those skilled in the art.

The invention further discloses oral pesticidal compositions comprising one or more agents as described above. Such compositions preferably further comprise other pesticidal materials from non-*Xenorhabdus* species.

These other materials may be chosen such as to have complementary properties to the agents described above, or act synergistically with it.

Preferably the oral pesticidal composition comprises one or more pesticidal agents as described above in combination with *B. thuringiensis* (or with a toxin derived therefrom, preferably endotoxin).

Recombinant DNA encoding said proteins also forms a further aspect of the invention. The DNA may be incorporated into an expression vector under the influence of suitable control elements such as promoters, enhancers, signal sequences etc. as is understood in the art. These expression vectors form a further aspect of the invention. They may be used to transform a host organism so as to ensure that the organism produces the toxin.

The invention further makes available a host organism comprising a nucleotide sequence coding for a pesticial agent as described above.

Methods of cloning the sequence for a characterised protein into a host organism are well known in the art. For instance the protein may be purified and sequenced: as activity is not required for sequencing, SDS gel electrophoresis followed by blotting of the gel may be used to purify the protein. The protein sequence can be used to generate a nucleotide probe which can itself be used to identify suitable genomic fragments from a *Xenorhabdus* gene library. These fragments can then be inserted via a suitable vector into a host organism which can express the protein. The use of such general methodology is routine and non-inventive to those skilled in the art. Such techniques may be applied to the production of *Xenorhabdus* toxins other than those encoded by the sequence of Figure 2.

It may be desirable to manipulate (eg. mutate) the agent by altering its gene sequence (and hence protein structure) such as to optimise its physical or toxicological properties.

It may also be desirable for the host to be engineered or selected such that it also expresses other proteinaceous pesticidal materials (eg. delta- endotoxin from *B. thuringiensis*). Equally it may be desirable to generate host organisms which express fusion proteins composed of the active portion of the agent plus these other toxicity enhancing materials.

A host may be selected for the purposes of generating large quantities of pesticidal materials for purification e.g. by using *B.thuringiensis* transformed with the agent-coding gene. Preferably however the host is a plant, which would thereby gain improved pest-resistance. Suitable plant vectors, eg. the Ti plasmid from *Agrobacterium tumefaciens*, are well known in the art. Alternatively the host may be selected such as to be directly pathogenic to pests, eg. an insect baculovirus.

The teaching and scope of the present invention embraces all of these host organisms plus the agents, mutated agents or agent-fusion materials which they express.

Thus the invention makes available methods, compositions, agents and organisms having industrially applicable pesticidal activity, being particularly suited to improved crop protection or insect-mediated disease control.

The methods, compositions and agents of the present invention will now be described, by way of illustration only, through reference to the following non-limiting examples and figures. Other embodiments falling within

the scope of the invention will occur to those skilled in the art in the light of these.

FIGURE

Figure 1 shows the variation with time of the growth of *X. nematophilus* ATCC 19061 and activity of cells and supernatants against *P. brassicae* as described in Example 3.

Figure 2 shows the sequence of a major part of a cloned toxin gene from *Xenorhabdus*.

Figure 3 shows a comparison of the restriction maps of cloned toxin genes from two strains of *Xenorhabdus* (clone 1 above and clone 3 below).

EXAMPLES

Example 1 - Use of *X. nematophilus* cells as an oral insecticide

CELL GROWTH: A subculture of *X.nematophilus* (ATCC 19061, Strain 9965 available from the National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland) was used to inoculate 250 ml Erlenmeyer flasks each containing 50 ml of Luria Broth containing 10g tryptone, 5g yeast extract and 5g NaCl per litre. Cultures were grown in the flasks at 27°C for 40hrs on a rotary shaker.

PRODUCTION OF CELL SUSPENSION: Cultures were centrifuged at 5000 x g for 10 mins. The supernatants were discarded and the cell pellets washed once and resuspended in an equal volume of phosphate buffered saline (8g NaCl, 1.44g Na_2HPO_4 and 0.24g of KH_2PO_4 per litre) at pH 7.4.

ACTIVITY OF CELL SUSPENSION TO INSECTS: The bioassays were as follows: *P. brassicae*: The larvae were allowed to feed on an artificial agar-based diet (as described by David and Gardiner (1965) London Nature, 207, 882-883) into which a series of dilutions of cell suspension had been incorporated. The bioassays were performed using a series of 5 doses with a minimum of 25 larvae per dose. Untreated and heat-treated (55°C for 10 minutes) cells were tested. Mortality was recorded after 2 and 4 days with the temperature maintained at 25°C.

	LC50 cells/g diet	
Treatment	2 days	4 days
Untreated	5.9×10^5	9.8×10^4
Treated 55°C	7.1×10^5	1.4×10^5

Aedes aegypti: The larva were exposed to a series of 5 different dilutions of cell suspension in deionised water. The biosassays were performed using 2 doses per dilution of 50 ml cell suspension in 9.5cm plastic cups with 25 second instar larvae per dose. Untreated and heat-treated (55°C or 80°C for 10 minutes) cells were tested. Mortality was recorded after 2 days with the temperature maintained at 25°C.

	LC50 cells/ml
Treatment	2 days
Untreated	5.1×10^6
Treated 55°C	7.4×10^6
Treated 80°C	$> 10^8$

Culex quinquefaciatus: The larvae were exposed to a single concentration cell suspension containing 4×10^7 cells/ml. The biosassays were performed using 2 50 ml cell suspensions in 9.5 cm plastic cups with 25 second instar larvae per cup. Untreated and heat-treated (55°C or 80°C for 10 minutes) cells were tested. Mortality was

recorded after 2 days with the temperature maintained at 25°C.

Treatment	% Mortality 2 days
Untreated	100
Treated 55°C	100
Treated 80°C	0

Thus these results clearly show that cells from *X. nematophilus* are effective as an oral insecticide against a number of insect species (and are particularly potent against *P.brassicae*). The insecticidal activity is not dependent on cell viability (i.e is largely unaffected by heating to 55°C which reduces cell viability by >99.99%) but is much reduced by heating to 80°C, which denatures most proteins.

Example 2 - Use of *X.nematophilus* supernatant as an oral insecticide

CELL GROWTH: Cultures were grown as in Example 1.

PRODUCTION OF SUPERNATANT: Cultures were centrifuged twice at 10000g for 10 mins. The cell pellets were discarded.

ACTIVITY OF SUPERNATANT TO INSECTS: The Bioassay was as follows:
Activity against neonate *P. brassicae* and two day old *Pieris rapae* and *Plutella xylostella* larvae was measured as for *P. brassicae* in Example 1, but using a series of untreated dilutions of supernatant in place of of cell supensions and with mortality being recorded after 4 days only.

Insect species	LC50 (μl supernatant/g diet) 4 days
P. brassicae	22
P. rapae	79
P. xylostella	135

In addition, size-reducing activity (62% reduction in 7 days) against *Mamestra brassicae* was detected in larvae fed on an artificial diet containing *X. nematophilus* supernatant (results not shown).

Thus these results clearly show that the supernatant from *X. nematophilus* culture medium is effective as an oral insecticide against a number of insect species, and are particularly potent against *P. brassicae*.

The heating of supernatants to 55°C for 10 minutes caused a partial loss of activity while 80°C caused complete loss of activity. Activity was also completely lost by treatment with SDS (0.1%w/v for 60 mins) and Acetone (50% v/v for 60 mins) but was unaffected by Triton X-100 (0.1% 60 mins), non-diet P40 (0.1% 60 mins), NaCl (1 M for 60 mins) or cold storage at 4°C or -20°C for 2 weeks. All of these properties are consistent with a proteinaceous agent.

The general mode of action of *X. nematophilus* cells and supernatants i.e. reduction in larval size and death within 2 days at high dosages, and other properties, eg. temperature resistence, appear to be similar suggesting a single agent or type of agent may be responsible for the oral insecticide activity activities of both cells and supernatants.

Example 3 - Timescale for appearance of ingestable insecticidal activity

CELL GROWTH: 1ml of an overnight culture of *X. nematophilus* was used to inoculate an Erlenmeyer flask. Cells were then cultured as in Example 1. Growth was estimated by measuring the optical density at 600 nm.

PRODUCTION OF CELL SUSPENSION AND SUPERNATANTS: These were produced as in Examples 1 and 2.

ACTIVITY OF CELLS AND SUPERNATANTS AGAINST *P. BRASSICAE*: The cell suspension bioassay was carried out as in Example 1, but using a single dose of suspended cells equivalent to 50 μl of broth/g diet and measuring mortality after 2 days. The cell supernatant bioassay was carried out as in Example 2, but using a single dose equivalent to 50 μl supernatant/g diet (i.e. more than twice the LC50) and measuring mortality after 2 days.

The results are shown in Fig. 1. Thus these results clearly show that cells taken from *X. nematophilus* culture medium are highly effective as an oral insecticide against *P. brassicae* after only 5 hours, and supernatants are highly effective after 20 hours. Although some slight cell lysis was observed in the early stages of growth, no significant cell lysis was observed after this point demonstrating that the supernatant activity may be due to an authentic extracellular agent (as opposed to one released only after cell breakdown).

Example 4 - Synergy between *X. nematophilus* cells and *B.thuringiensis* powder preparations

CELL GROWTH AND SUSPENSION: *X. nematophilus* cells were grown and suspended as in Example 1. *B. thuringiensis* strain HD1 (from *Bacillus* Genetic Stock Centre, The Ohio State University, Columbus, Ohio 43210, USA) was cultured, harvested and formulated into a powder as described by Dulmage *et al.*(1970) J. Invertebrate Pathology 15, 15-20.

ACTIVITY OF *X. NEMATOPHILUS* CELLS AND *B. THURINGIENSIS* POWDER AGAINST *P. BRASSICAE*: The bioassays was carried out using *X. nematophilus* and *B. thuringiensis* in combination or using *B. thuringiensis* cell powder alone. Bioassays were carried out as in Example 1 but with various dilutions of *B. thuringiensis* powder in place of *X. nematophilus*. For the combination experiment, a constant dose of *X. nematophilus* cell suspension sufficient to give 25% mortaility was also added to the diet. Mortality was recorded after 2 days.

Bioassay	LC50 (μg Bt powder/g diet) 2 days
B.t. alone	1.7
B.t. plus *X.nematophilus*	0.09

These results clearly demonstrate the synergism between *X. nematophilus* cells and *B. thuringiensis* powder when acting as an oral insecticide against *P. brassicae*.

Example 5 - Synergy between of *X.nematophilus* supernatants and *B. thuringiensis* powder

CELL GROWTH AND PRODUCTION OF SUPERNATANTS: *X. nematophilus* cells were grown and supernatants prepared as in Example 2. *B. thuringiensis* was grown and treated as in Example 4.

ACTIVITY OF *X. NEMATOPHILUS* SUPERNATANTS AND Bt CELL POWDER AGAINST *P. BRASSICAE*:
The bioassays were carried out using *X. nematophilus* supernatants and *B. thuringiensis* in combination or using *B. thuringiensis* powder alone. The Bioassay against neonate *P. brassicae* and two day old *Pieris rapae* and *Plutella xylostella* larvae were measured as in Example 2 but with various dilutions of *B. thuringiensis* in place of *X. nematophilus*. For the combination experiment, a

constant dose of *X. nematophilus* supernatant sufficient to give 25% mortality was also added to the diet. Mortality was recorded after 4 days.

	LC50 (µg Bt powder/g) diet	
Insect species	Bt alone	Bt plus Xn
P. brassicae	1.4	0.12
P. rapae	2.5	0.26
P. xylostella	7.2	0.63

These results clearly demonstrate the synergism between *X.nematophilus* supernatants and *B.thuringiensis* powder when acting as an oral insecticide against several insect species. The fact that both *X. nematophilus* cells and supernatants demonstrate this synergism strongly suggests that a single agent or type of agent is responsible for the demonstrated activities.

Example 5 - Characterisation of insecticidal agent from *X.nematophilus* supernatant by proteolysis

CELL GROWTH AND PRODUCTION OF SUPERNATANTS: *X. nematophilus* cells were grown and supernatants prepared as in Example 2.

PROTEOLYSIS OF SUPERNATANT: Culture supernatant (50ml) was dialysed against 0.5 M NaCl (3 x 1 l) for 48 hours at 4°C. The volume of the supernatant in the dialysis tube was reduced five-fold by covering with polyethylene glycol 8000 (Sigma chemicals). Samples were removed and treated with either trypsin (Sigma T8253 = 10,000 units/mg) or proteinase K (Sigma P0390 = 10 units/mg) at a concentration of 0.1 mg protease/ml sample for 2 hours at 30°C.

ACTIVITY OF PROTEASE TREATED SUPERNATANT AGAINST *P. BRASSICAE*: The boassay against neonate *P. brassicae*

larvae was carried out by spreading 25 μl of each 'treatment' on the artificial agar-based diet referred to in Example 1 in a 4.5 cm diameter plastic pot. Four pots each containing 10 larvae were used for each treatment. Mortalities were recorded after 1 and 2 days. Controls using water only, trypsin (0.1 mg/ml) and proteinase K (0.1 mg/ml) were also tested in the same way.

	% Mortality	
Treatment	1 day	2 days
Untreated supernatant	60	100
Proteinase K treated supernatant	45	100
Trypsin treated supernatant	40	100
All controls (no supernatant)	0	0

Example 6

Entomocidal activity of other *Xenorhabdus*

Using the methodology of Examples 1 and 2, four different *xenorhabdus* strains were tested against insect pests. The results obtained were as follows:

I) Activity to *Pieris brassicae*

Strain deposit no/code	Cells 10^6/grm diet % mortality	Supernatant LC50 μl/gram of diet
NCIMB 40887	100	0.09
0014	100	0.52
0015	80	3.73
NCIMB 40886	100	0.05

It was found that entomocidal activity of cells and supernatant was reduced by more than 99% when all four strains were heated at 80°C for 10 minutes.

II) Activity to mosquitoes (*Aedes aegypti*)
Bacteria added at the rate of 10^7cells/ml of water

Strain deposit no/code	Cells 10^6/grm diet % mortality
NCIMB 40887	0
0014	40
0015	45
NCIMB 40886	95

Furthermore, all strains significantly reduced the growth of *Heliothis virescens*.

Example 7

Cloning of toxin genes from strains of *Xenorhabdus*

Total cellular DNA was isolated from NCIMB 40887 and ATCC 19061 using a Quiagen genomic purification DNA kit. Cells were grown in L borth (10g tryptone, 5g yeast extract and 5g NaCl per l) at 28°C with shaking (150rpm) to an optical density of 1.5 A_{600}. Cultures were harvested by centrifugation at 4000xg and resuspended in 3.5mls of buffer B1 (50mM Tris/HCl, 0.05% Tween 20, 0.5% Triton X-100, pH7.0) and incubated for 30 mins at 50°C. DNA was isolated from bacterial lysates using Quiagen 100/G tips as per manufacturers instructions. The resulting purified DNA was stored at -20°C in TE buffer (10mM Tris, 1mM EDTA, pH 8.0).

A representative DNA library was produced using total DNA of NCIMB 40887 and ATTC 19061 partially digested with the restriction enzyme *Sau3a*. Approximately 20µg of DNA from each strain was incubated at 37°C with 0.25 units of the enzyme. At time intervals of 10, 20, 30, 45 and 60 minutes, samples were withdrawn and heated at 65°C for 15 minutes. To visualise the size of the DNA fragments, the samples were electrophoresed on 0.5% w/v agarose gels.

The DNA samples which contained the highest proportion of 30 to 50kb fragments were combined and treated with 4 units of shrimp alkaline phosphatase (Boehringer) for 15 minutes at 37°C, followed by heat treatment at 65°C to inactivate the phosphatase.

The size selected DNA fragments were ligated into the BamH1 site of the cosmid vector SuperCos! (Stratagent) and packaged into the *Escherichia coli* strain XL Blue 1, using a Gigapack II packaging kit (Stratgene) in accordance with the manufacturers instructions.

To select for cosmid clones with entomocidal activity, individual colonies selected on L agar plates containing 25μg/ml ampicillin, were grown in L broth (containing 25μg/ml ampicillin) overnight at 28°C. Broth cultures (50μl) were individually spread onto the surface of insect diet contained in 4.5cm diameter pots, as described in Example 5. To each container 10 neonate *P. brassicae* larvae were added. Larvae were examined after 24, 72 and 96 hours recording mortality and size of surviving larvae. A total of 220 clones of NCIMB 40887 were tested, of which two were found to cause reduction in larval growth and death within 72 hours. Of 370 clones from ATTC 19061, one was found to cause larval death within 72 hours.

Example 8

<u>Activity of cloned toxin genes to *Pieris brassicae*</u>

The three active clones from Example 7 were grown in L broth, containing 25μg/ml ampicillin, for 24 hours at 28°C, on a rotary shaker at 150rpm. The activity of the toxin clones to neonate larvae were performed by incorporation of whole broth cultures into insect diet, as described in Example 1.

Clone No	Strain	LC50 (µl broth/g insect diet)
1	NCIMB 40887	13.03
2	NCIMB 40887	16.7
3	ATTC 19061	108.7
Control*		No effect at 100µl/g

*XL1 Blue *E. coli* broth

When *E. coli* toxin clones were heated at 80°C for 10 minutes and added to the diet at a rate of 100µl/g, no activity to larvae was detected. Highlighting the heat sensitivity of the toxins.

Example 9

Sequencing of the cloned toxin from NCIMB 40887

Cosmid DNA of the entomocidal clone 1 above from NCIMB 40887 was purified using the Wizard Plus SV DNA system (Promega) in accordance with the manufacturers instructions. A partial map of the cloned fragment was obtained using a range of restriction enzymes *Eco*R1, *Bam*H1, *Hind*III, *Sal*1 and *Sac*1 as shown in Figure 3. DNA sequencing was intiatiated from pUC18 and pUC19 based sub-clones of the cosmid, using the enzymes *Eco*R1, *Bam*H1, *Hind*III, *Eco*RV and *Pvu*II. Sequence gaps were filled using a primer walking approach on purified cosmid DNA. Sequence reactions were performed using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmmpliTaq DNA polymerase FS according to the manufacturers instructions. The samples were analysed on an ABI automated sequencer according to the manufacturers instructions. The major part of the DNA sequence for the cloned toxin fragment is shown in Figure 2.

Example 10

Restriction map of cloned toxin from clone 3

Cosmid DNA of the entomocidal clone 3 above was purified as described in Example 9. A restriction map of the cloned fragment was obtained using the restriction enzymes *Bam*H1, *Hind*III, *Sal*1 and *Sac*1 and this is shown in Figure 3. When compared with the map from clone 1 (Figure 3) it is clear that over the regions which overlap, the restriction maps are very similar. The only detectable difference between the two clones was a reduction in size of two *Hind*III fragments in clone 3, corresponding to the 11.4kb and 7.2kb *Hind*III fragments in clone 1 by approximately 2Kb and 200bp respectively. These results indicate the overall relatedness of the DNA region coding for toxicity in the two bacterial strains.

Example 11

Southern Blot Hybridisation Experiments

A 10.3kb *Bam*H1-*Sal*1 fragment of the DNA from clone 1 was used as a probe to hybidise to total *Hind*III digested DNA of the *Xenorhabdus* strains ATCC 19061, NCIMB 40886 and NCIMB 40887. Hybridisation was performed with 20ng/ml of DIG labelled DNA probe at 65°C for 18 hours. Filters were washed prior to immunological detection twice for 5 minutes with 2 x SSC (0.3M NaCl, 30mM sodium citrate, pH 7.0)/0.1% (w/v) sodium dodecyl sulphate at room temperature, and twice for 15 minutes with 0.1 x SSC (15mM NaClm 1.5 mM sodium citrate, pH 7.0) plus 0.1% sodium dodecyl sulphate at 65°C. The probe was labelled and experiments performed in accordance with manufacturers instructions, using a non-radioactive DIG DNA labelling and detection kit (Boehringer). The probe hybridised to a *Hind*III fragment of approximately 8kb in all three strains as well as an 11.4kb fragment in NCIMB 40887 and an approximate 9kb fragment in both NCIMB 40886 and ATCC 19061. These results show that strains NCIMB

40886 and ATCC 19061 contain DNA with close homology to the toxin gene of clone 1 above, confirming the similarity between the toxins produced by the three strains.

CLAIMS

1. An insecticidal composition adapted for oral administration to an insect comprising a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.

2. A composition according to claim 1 wherein the said pesticidal material comprises material encoded by the nucleotide sequence of Figure 2 or variant or fragment thereof, or a sequence which hybridises with said sequence.

3. A composition according to claim 1 or claim 2 which comprises cells of *Xenorhabdus*.

4. A composition as claimed in any one of the preceding claims which comprises supernatant taken from cultures of cells of *Xenorhabdus* species.

5. A composition according to any one of the preceding claims wherein the *Xenorhabdus* species is *Xenorhabdus nematophilus*.

6. A composition according to any one of claims 1 to 4 wherein the *Xenorhabdus* species is ATCC 19061, NCIMB 40886 or NCIMB 40887.

7. A composition as claimed in any one of the preceding claims which comprises a further pesticidal material not obtainable from *Xenorhabdus*.

8. A composition according to claim 7 wherein the said further pesticidal material comprises a material obtainable from *B. thuringiensis*.

9. A composition according to claim 8 which further comprises cells of *B. thuringiensis*.

10. A composition according to claim 8 wherein the pesticidal materials obtainable from *B.thuringiensis* comprises the delta endotoxin.

11. A composition according to any one of the preceding claims which further comprises an agriculturally acceptable carrier.

12. A composition according to claim 10 wherein the carrier comprises items of insect diet.

13. A method for killing or controlling insect pests, which method comprises administering to a pest or the environment thereof a composition according to any one of the preceding claims.

14. A method as claimed in claim 12 wherein the pests are insects from the order Lepidoptera or Diptera.

15. A microorganism comprising *Xenorhabdus* strain NCIMB 40886.

16. A microorganism comprising *Xenorhabdus* strain NCIMB 40887.

17. A pesticidal agent which comprises a a toxin comprising a protein which is encoded by DNA which includes SEQ ID No. 1 or a variant or fragment thereof.

18. An isolated pesticidal agent characterised in that it is obtainable from cultures of *X. nematophilus* or mutants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55°C, is proteinaceous, acts synergistically with *B. thuringiensis* cells as an

oral pesticide, and is substantially resistant to proteolysis by trypsin and proteinase K.

19. An isolated pesticidal agent as claimed in claim 18 further characterised in that the pesticidal activity is substantially destroyed by treatment with sodium dodecyl sulphate or acetone or heating to 80°C.

20. An isolated pesticidal agent as claimed in claim 18 or claim 19 further characterised in that the agent is an extracellular protein.

21. A recombinant DNA which encodes a pesticidal agent according to any one of claims 17 to 20.

22. A recombinant DNA of claim 21 which comprises the sequence of Figure 2 or a variant or fragment thereof.

23. A recombinant DNA which comprises or hybridises under stringent conditions with all or part of the sequence of Figure 2, and which encodes a pesticidal material.

24. An expression vector comprising a recombinant DNA according to any one of claims 21 to 23.

25. A host organism which has been transformed with an expression vector according to claim 24.

26. A host organism as claimed in claim 25 which has been engineered or selected such that it also expresses other pesticidal proteinaceous toxicity enhancing materials

27. A host organism comprising a nucleotide sequence coding for a fusion protein comprising a pesticidally active portion of an agent as claimed in any one of claims 17 to 20 in combination with other pesticidal proteinaceous toxicity enhancing materials.

28. A host organism as claimed in claim 27 wherein the pesticidal toxicity enhancing materials comprise delta-endotoxin from B. thuringiensis.

29. A host organism as claimed in any one of claims 25 to 289 wherein the host is a plant.

30. A host organism as claimed in any one of claims 25 to 28 wherein the host is a virus pathogenic to insects.

31. A fusion protein as expressed by a host as claimed in claim 27.

32. An pesticidal composition comprising one or more agents as claimed in any one of claims 17 to 20.

Fig.1.

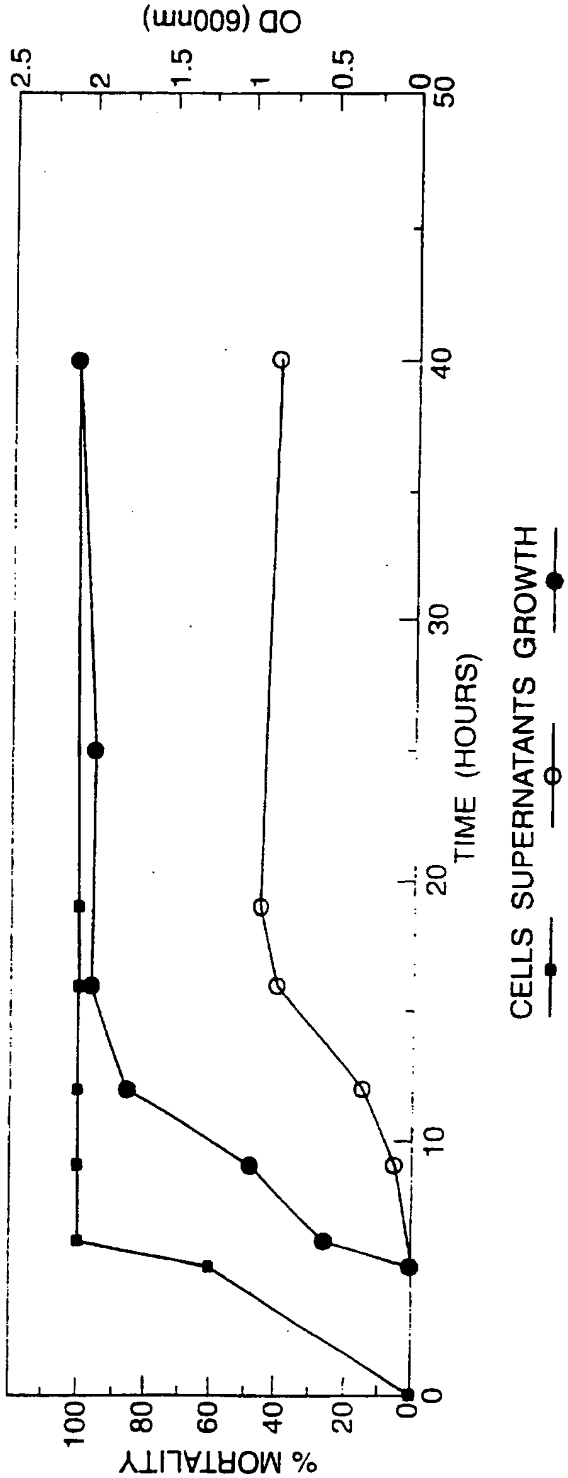
OD (600nm)
2.5
2
1.5
1
0.5
0
% MORTALITY
100
80
60
40
20
0
TIME (HOURS)
0
10
20
30
40
50
CELLS
SUPERNATANTS
GROWTH

Fig.2.

1	TCCACAATTG	CCGGAGAAAA	TCAGTCGGGA	ACTGCCGGTG	ATTATTCGTC	ACTTATTAAA
61	CGAATTTGCC	GACCAGAATA	AGGCTAAAAA	ACTGCTACAG	GCGCAACGCG	ACTCGAACGA
121	AGCGTTAACG	GTAAAGAGTC	ATTCGGATCC	GCTGTATCGC	TTTTGTGGTT	ATCTGGTGTC
181	TGTCAATGAT	ATGACCGGAA	TGAAGATGGG	CAATAAAAAC	ATTAGCCCAC	GAGCACCGAG
241	ATTGTACTTG	TATCATGCCT	ATCTCTCTTT	TATGGAAGCG	CACGGCTTTG	AACGTCCGTT
301	AACACTGACT	AAGTTTGGTG	AATCCATCCC	CAAGATTATG	CTGGAATACC	GGAAGGAGTA
361	TCGAAAAGTG	CGAACCAAGA	AAGGCTATTC	CTATAACGTG	GAATTATCGG	AAGAGGCCGA
421	AGAATGGCTA	CCGTCAGTGC	CTGAGTGTCG	AGACTTTAAA	TCACCTGTAT	AAAACTTTGA
481	GCTTTAAGTC	TGCACTCCAT	ACACAACTTA	AAATATCTAA	TTGTATTTAA	AAGAAAATAA
541	TAGATGTATA	GTTATTTTTT	AACTATACAT	AAGCTCTACA	TGCTCTTCAT	TCGTGTAAAA
601	AATGGGTGAA	CAGGTGATAC	AGTCAGTGAA	TATCATATTA	ATTACCGTAA	ACCCAGATGT
661	AGCAAGGCTT	TCAGGGAATT	GTGCAGAGGG	TGCATAACTG	AGAGGGTGAA	AAAGATTTTC
721	AGGGGGGCTT	ATGGCAGGTA	AACAAAATCA	GAAGCAAATA	CCGTGCACAA	TCTGGTTTTT
781	ATTTTTTGGT	ACTACCTCAA	ATTAAAATGA	TGTAATCATC	TGATTTTATT	TAAGAATAGA
841	AGTTAATCAC	AATTTCATTG	ATGGACTTTC	ATTCACACTG	GTATAGATAA	ATAATTCTGT
901	TATATCCTGT	TTCATTACGC	ATTCATCAGG	AGTGCTGTTA	CAGGAGACAA	GAATGTCACA
961	CATCATTTAC	TTGTCGTTAA	AGGGCAAGAA	GCAGGGTTTA	ATTTCAGCGG	GTTGTTCAAC
1021	GCCTGAATCA	ATTGGAAATC	GCTATCAAAA	AGGACGTGAA	GATCAAATAC	AGGTATTGAG
1081	CCTGAATCAT	TCGATGAGCC	GTGACCAGAA	TGTTAATCAT	CAACCCGTCA	GTTTTGTGAA
1141	ACCCATTGAT	AAATCCTCTC	CCCTGTTTGC	TGGATGCCAG	TTTTGTGCAT	TACAGGACAA
1201	GCCAGATGGG	ACAACTGGAG	TTCTTTTATG	AAATCAAGCT	GACCAGTGCC	ACGATTGTGG
1261	ATATTTCCTA	TAATTATCCG	GCATTCAATC	AATGATAATG	GTGCGATACC	CCATGAAGTG
1321	GTGATGCTCG	ATTATAAGTC	CATTTCATGC	AACCACATCG	CCGCAGGACT	TCGGGCTACA
1381	GCATACGCAA	TTAGCCGGAA	GTGAAGAAGC	AAGCCGCTTT	TATCTGGGGT	CTCGAATGTT
1441	AAGCCACTTA	AGAAGCCGCT	GGTTGAAGAA	ACCCCGGTAA	AACCCGCTAA	ACATCATGCC
1501	CGTTATCGTT	GTGTGGATGA	TGACGGCAAT	CTTTTAACCG	AACGCAAGTA	TCGGGTTTGC
1561	CTGCCGGATG	GTCAGATAAA	AGAAGGAAAG	ACTGATAAAC	AAGGTTACAC	CCAATGGCAT
1621	CTTACGGATG	ACAAAAATAA	ACTTGAATTT	CATATTTTAA	AGGATTAATA	CCATGCCAGC
1681	CTATACCGTT	CAGACAAAAA	TAGAATCCAA	CGTACCTGTT	GAAAACCTGC	TTTACGACTT
1741	AACCATTTAT	CGTAAGGATG	CAAAAGGAAA	TTTCCATATC	TTGCTTGATG	TTTTTCAGGA
1801	GAAACTACAG	AGTAATTATG	AAACACAACA	GCATATCACG	CAGGAAATAG	ACGACGATCT
1861	TTCTGTGATT	TATATTATGC	AAATTATGCT	TCACCGCAAA	CATGGCTCAA	ATATATTTCC
1921	GGCACTGCAA	ACCCATTTTA	AGAAAATGTA	TACCCTCGGT	GAATTAACTT	CCGGTAAAGC
1981	CTGTTCGGAG	AAAAAACGGG	AAAATGCCTG	TTATTTTGAA	AGTACAGTTG	AAACAAAACC
2041	TGTCAGCGAC	GGGGATAATA	CCGTTGACTT	AAATATCACT	ATTCCTGAAC	GACCTTTTAT
2101	TGCCAAAGAA	TATCCCATTG	GTCACCCACA	CGATCCATTT	GAAAAAAGTA	AAATTGAATC
2161	ATAAATACAG	GACAGGTTAT	CGAAAAGAAT	TTATCCGGAT	CAAAATGGAG	CAAGTTTATG
2221	TCAGGGCGCG	AGCACACTAT	TTTAGCTGCG	TTTTTAAGAT	GATTATCTCT	TAATGTTCAG
2281	TTTTAATAGT	GTTTTTATCG	AGTGAAATTT	AATCGCACAG	GCAATTCTTT	AGACTTTTAT
2341	AGAAAACTAA	AGAATTAAAG	AACAAGATTG	ACATTTTAAG	TTCAAATATT	AATCAAAGTA
2401	TGCTCGCGCC	CTGAGTTTAT	GTGGCCCTGC	CGCTTTTTTT	TATTGCCTGC	CAATAGATAG
2461	ACCAGATATT	TATGAGCAAG	CGGCACGAGA	ATTATGGCAA	TATGGCCGAA	CTAAAATTGG
2521	TCAACTGGAA	ATTAAGCCGG	GTGAGGGTTG	CCGACATCCT	AAAGGTACTT	TTTATAATCA
2581	ATATGGTGAA	AGAATATCTG	GGTTAGATTG	GCTGACATTG	GCAAGCCTAA	GAGATTCAGA
2641	AAATATGATG	ATGAGGTTGA	TGATGAAGTA	GCTGGTATTA	CAATGTGGGG	AAAATTGACA
2701	GAATGGTTTG	AAAAATCAGG	GTATGAAAAA	GTATTTAGTA	ATGTCGGCTT	ATCCCATTCT
2761	AATATAAATG	ACATAGTAAC	TCTTAGTGAT	TACTATAACA	AAGGATATCA	TGTTGTTACT
2821	TTGATTTCAG	CAGGAATGTT	ATCAGATTTT	GGTGACATAG	AAACATCAGG	AAAAAATCAT
2881	TGGATAGTTT	GGGAAGGAGT	AGTAGAAAAC	TATGAGAAAG	AAAATATCAC	AAATAATTCA
2941	GATCTGAATC	AATATGTAAA	TTTAAATCTG	TTTTCATGGG	GTAAAGTGGA	ACATCAAATT
3001	AAAAAAAACA	AATCACTAGA	TTATGTACTC	AACCATATTT	TTTGAGGGTT	GGTTTTTAAA
3061	CCAATGAAAT	AACATGAAAA	AAATATTAAT	TATTTTTATT	TTTTTACTTT	ATGGTTGTGG
3121	TAATCCAACG	CCAAAAGTTT	TACCAAAATC	AGAGTTTCTT	CCTGATGCAG	TGATAAATGA
3181	ACCATATCAG	GCATCAATTA	CCATCACAGG	AGGTGCATTG	AATGAAAAAA	GCGTTTGGGT
3241	AAAAATTCAT	CCTACTGGCT	CAGGACTAAC	ATGGAATCCA	AAAGATAGTT	CTTTCCTATA
3301	GGGTGGAAAA	AAAGAAATAA	GAAAAGATTA	TCATCATATA	AATATAACAG	GTACCCCAAA
3361	GAAGACAGAA	TTGATAAAAA	TTGAAGTGGT	AGGATTTACA	TTGGGTACAA	TGTACGCACG
3421	GAAAGAGTTC	ACTATAAATT	ATACTATAAA	AGTAAGGGAA	TAATTGTCAC	TATCAGAATG
3481	GTGATTTAAT	TCGCCATTTT	TATACTTTTG	TATACTCTCT	CAACATAATC	AGGATTCTTT

Fig.2.

3541 CTTATTATTT TTCATGGTGC TAAAAACGTT TATTGCAAAA ATAAATTAAG TTAATCAGAT
3601 AAATTATCTG CATTACTGTT ATAATCGATA ACACGATAAC CTGACTTTCT GCCTGTTCTT
3661 ATGAACTCGA AGATAATCCT TTCTGAGCCT GAACGAATCA CATTGCAACC ACTCGCTTTG
3721 AATCACCCAC ACCGGGACAT TCGTACGCGA GGAACGGGTT TACTCATGCT TGCCAGAGGG
3781 AGCAAGCCGT CCCAGATCAC CGCTGAAATC GGATGCAGTC TCCGGGTTAT CTGTAATTGG
3841 GTTCACATGT GGCACAGATA GCGGGATTAT TCGGCGGTCA TGCCGGAGGC CGGTATCTCG
3901 CCATGACGCC TGACATGATT GCCACTGCGC TCGAAGCCGC CAGCGCAGAG TCCCTGACGT
3961 GCGTCGAAGC CAGGCAGGGT TTCCCTGCCT TGTACGCTTG AAACGCTGGC GAATACCCTG
4021 AAAAAACAGG GGCTCCCCTA TAAACGCCCC CGCCTGTCGC TTAAAAAAAG CGCAATAAAA
4081 CGGAGTTTGC TGAAAAATCC GCCTTGCTGA ATAAAATTAA GGCCGGAGCA CAGTCAGGAC
4141 ATTACCGTCT GGTCTATTTT GAGTTCTGGG GGCGTTAAAT TACACGGATA ACACGCTGTT
4201 TTACCAGACA ACGTCAGGCA GTATCACGCG AGATGACGTG ATTGATTTTT TAGAGCCGGT
4261 GGCCAGACAA GGGACAACCG CCTGACATTT TTAGTGTTGG ATAATGCGCG TATCCATCAC
4321 GGGATAGAGG AAAAAATCAG AAATGGCGGG TGACGAGAAC ACAACCTGTT TTTATTCTAT
4381 CTTCCCGCTT ACAGCCCAGA GCTGTATCTG ATTGAAATCG TCTGGAAACA GGCCAAATAC
4441 GACTGGCGAC GTTTTATCAC CTGGACTCAG GATACAATGG AATATGAGGT AAATACTTTA
4501 TTGAAAGGTT ATGGCGACCA ATTTGCAATT AACTTTTCTT GAGTACTTAG TAAGAATAGA
4561 GTCAGTCGAG GTTTTTTCAT TTCGGGTCGT GGGGATGATA CTGAAAATTT GTTTGTAATC
4621 TCTGAAAATT GCTGTTTCTG TGGCTACGTC TGTCTTTTGG GATATTGTTT CCATCAAGTC
4681 TGTCAACATA CTGTTAAGTT AGATGTTGAT AAAAGAGACT GAATTATAAT ACAAAACAAT
4741 AAATCACTTG GACAATATTT TATTTCACAT GAGACATTAA GGTTGATTTT CCCAATCTGG
4801 TCAGTTATAA CCGAATAAGG ATCTTGAAAA ATCATGGGAT CTTACTTTTA TCAAATGAAG
4861 TTAACGTAAA AGTTGATAAA GAAAATTATT TAATTCTAAG TGCCGTTGGC ATAAATATTT
4921 TGTGTTTTGT TAATGAATGA ATAACCAGGT AAGCTGGATT TTCATTTTTT AATTACTCGT
4981 TACAATATGC TATTTATTTA TATAAAGAGT TTGTGCCCAT TTAACCAGTA AACAAATTTG
5041 TTCAACCGTA ACTTAGCTTC ATCGACTTTT GGCCTCGCCT GGTCAGAATC TAGGGCCGTT
5101 ATCCTATTTA TTTATGATAA ATAAAATTTA ATTATCTTTA ATAAGCTGAA TATGTGGATT
5161 TGTGCTCAAT CTTGGATTCA AGTATGTATT CCTTTTGGTA CCCTGCTTTA TTTTAAGGCA
5221 GATGAAGAGG ATGCCAACAT GACACAATAT CGATTACGAC TGTAACATTA AAGTCAGTTA
5281 TAAATTTTAT GATTAAAATG AAATTTTAGT AGAAAATCGT ATTCTATTCC GCCATTTACA
5341 ATAGCATCCT CTTTAATATC ATTAATCTCA GATAAAACAA ATAATTACAA TGTGAATAGA
5401 ATAATGACTT ACAAAATAAG CACTAAATCT TCAGATGAAC TCTTAACTGA CAACACTATT
5461 TTATAAAATA ATTGAGGTTA TTATGTATAG CACGGCTGTA TTACTCAATA AAATCAGTCC
5521 CACTCGCGAC GGTCAGACGA TGACTCTTGC GGATCTGCAA TATTTATCCT TCAGTGAACT
5581 GAGAAAAATC TTTGATGACC AGCTCAGTTG GGGAGAGGCT CGCCATCTCT ATCATGAAAC
5641 TATAGAGCAG AAAAAAAATA ATCGCTTGCT GGAAGCGCGT ATTTTTACCC GTGCCAACCC
5701 ACAATTATCC GGTGCTATCC GACTCGGTAT TGAACGAGAC AGCGTTTCAC GCAGTTATGA
5761 TGAAATGTTT GGTGCCCGTT CTTCTTCCTT TGTGAAACCG GGTTCAGTGG CTTCCATGTT
5821 TTCACCGGCT GGCTATCTCA CCGAATTGTA TCGTGAAGCG AAGGACTTAC ATTTTTCAAG
5881 CTCTGCTTAT CATCTTGATA ATCGCCGTCC GGATCTGGCT GATCTGACTC TGAGCCAGAG
5941 TAATATGGAT ACAGAAATTT CCACCCTGAC ACTGTCTAAC GAACTGTTGC TGGAGCTATT
6001 ACCCGCAAGA CCGGAGGTGA TTCGGACGCA TTGATGGAGA GCCTGTCAAC TTACCGTCAG
6061 GCCATTGATA CCCCTTACCA TCAGCCTTAC GAGACTATCC GTCAGGTCAT TATGACCCAT
6121 GACAGTACAC TGTCAGCGCT GTCCCGTAAT CCTGAGGTGA TGGGGCAGGC GGAAGGGGCT
6181 TCATTACTGG CGATTCTGGC CAATATTTCT CCAGAACTGT ATAACATTTT GACCGAAGAG
6241 ATTACGGAAA AGAACGCTGA TGCTTTATTT GCGCAAAACT TCAGTGAAAA TATCACGCCC
6301 GAAAATTTCG CGTCACAATC ATGGATAGCC AAGTATTATG GTCTTGAACT TTCTGAGGTG
6361 CAAAAATACC TCGGGATGTT GCAGAATGGC TATTCTGACA GCACCTCTGC TTATGTGGAT
6421 AATATCTCAA CGGGTTTAGT GGTCAATAAT GAAAGTAAAC TCGAAGCTTA CAAAATAACA
6481 CGTGTAAAAA CAGATGATTA TGATAAACAT GTAAATTACT TTGATCTGAT GTATGAAGGA
6541 AATAATCAAT TCTTTATATG TGCTAATTTT AAGATATCGA GAGAATTTGG GGCGACTCTT
6601 AGGAAAAACT CAGGGACAAG TGGCATTGTC GGCAGCCTTT CCGGTCCCCT GGTAGCCAAT
6661 ACTAATTTCA AAAGCAATTA CTTAAGTAAC ATATCTGATA ATGAATACAG AAATGGCGTA
6721 AAAATATATG CCTATCGCTA TACGTCTTCC ACCAGCGCCA CAAATCAGGG CGGCGGAATA
6781 TTCACTTTTG AGTCTTATCC CCTGACTATA TTTGCGCTCA AACTGAATAA AGCCATTCGC
6841 TTGTGCCTGA CTAGCGGGCT TTCACCGAAT GAACTGCAAA CTATCGTACG CAGTGACAAT
6901 GCACAAGGCA TCATCAACGA CTCCGTTCTG ACCAAAGTTT TCTATACTCT GTTCTACAGT
6961 CACCGTTATG CACTGAGCTT TGATGATGCA CAGGTACTGA ACGGATCGGT CATTAATCAA
7021 TATGCCCGAC GATGACAGTG TCAGTCATTT TAACCGTCTC TTTAATACCC CGCCGCTGAA
7081 AGGGAAAATC TTTGAAGCCG ACGGCAACAC GGTCAGCATT GATCCGGATG AAGAACAATC
7141 TACCTTTGCC CGTTCAGCCC TGATGCGTGG TCTGGGGATC AACAGTGGTG AACTGTATCA
7201 GTTAGGCAAA CTGGCGGGTG TATTGGACAC ACAAAATATC CTCACACTTT CTGTCCCTGT
7261 TATATCTTCA CTGTATCGCC TCACGTTACT GGCCCGTGCC CATCAGCTGA CGGTTAATGA
7321 ACTGTGTATG CTTTATGGTT TTTCGCCGTT CAATGGCAAA ACAACGGCTT CTTTGTCTTC

Fig.2.

7381 CGGGGAGTTG TCACGGCTGG TTATCTGGTT GTATCAGGTG ACGCAGTGGC TGACTGAGGG
7441 CGGAAATCAC CACTGAAGCG ATCTGGTTAT TATGTACGCC AGAGTTCAGC GGGAATATTT
7501 CACCGGAAAT CAGTAATCTG CTTAATACTC TCCGACCCCG TATTAGTGAA GACATGGCAC
7561 AAAGTAGTGA CCGGGAGCTT CAGGCTGAAA TTCTCGCGCC GTTTATTGCT GCAACGCTGC
7621 ATCTGGCGTC ACCAGATATG GCGCGGTATA TCCTGTTGTG GACTGATAAC CTGCGGCCGG
7681 GCGGCCTGAA TATCGCCGGA TTTATGATGC TGGTGCTGAA AGAGACGCTG AGTGATGAGG
7741 AAACGACCCA ACTGGTTCAA TTCTGCCATG TAATGGCACA GTTATCGCTT TCCGTGCAGA
7801 CACTGCGTCT CAGTGAAGCA GAGCTTTCTG TGCTGGTCAT TTCCGATTTT GTGGTACTGG
7861 GTGCGAGAAG CCAACCGCCG GACAACACAA TATTGATACT CTGTTCTCAC TCTACCGATT
7921 CCACCAGTGG ATTAATGGGC TGGGAAATCC CGGCTCTGAC ACGCTGGATA TGCTGCGCCA
7981 AGCAGACACT CACGGGCGAC AGACTGGGCC TCCGTGATGG GGCTGGACAT CAGTATGGTA
8041 ACGCAGGCCA TGGGTTCCCG CCGGCGTGAA CCAACTTCAG TGTTGGCAGG ATATCAACCC
8101 CGTGTTGCAG TGGATACATG TGGCATCAGC ACTGCTCACT GATGCCGTCG GTTATCCGTA
8161 CGCTGGTGAA TATCCGTTAC GTGACTGCAT TAAACAAAGC CGAGTCGAAT CTGCCTGCCT
8221 GGGATAAGTG GCAGACGCTG GCAGAAAATA TGGCAGCCGG ACTGAGTACA CAACAGGCTC
8281 AGACGCTGGC GGATTATACC GCAGAGCGCC TGAGTAACGT GTTGTGCAAT TGGTTTCTGG
8341 CGAATATCCA GCCAGAAGGG GTGTCCCTGC ACAGCCGGGA TGACCTGTAC AGCTATTTCC
8401 TGATTGATAA TCAGGTCTCT TCTGCCATAA AAACCACCCG ACTGGCAGAG GCCATTGCCG
8461 GTATTCAGCT CTACATCAAC CGGGCGCTGA ACCGGATAGA GCCTAATGCC CGTGCCGATG
8521 TGTCAACCCG CCAGTTTTTT ACCGACTGGA CGGTGAATAA CCGTTACAGC ACCTGGGGCG
8581 GGGTGTCGCG GCTGGTTTAT TATCCGGAAA ATTACATTGA CCCGACCCAG CGTATCGGGC
8641 AGACCCGGAT GATGGATGAA CTGCTGGAAG ATATCAGCCA GAGTCAGCTC AGCCGGGACA
8701 CGGTGGAAGA GGCCTTTAAA ACTTACCTGA CCGCTTTGAA ACCGTGGCAG ACCTGAAAGT
8761 TGTCAGCGCT ATCACCGACA ACGTCAACAG CAACACCGGA CTGACCTGGT TTGTCGGCCA
8821 AACGCGGGAG AACCTGCCGG AATATTACTG GCGTAACGTG CATATATCAC GGATGCAGGC
8881 GGGTGAACTG GCCGCCGATG CCTGGAAAGA TTGGACGAAG ATTGATACAG CGGTCAACCC
8941 ATACAAGGAT GCAATACGTC CGGTCATATT CAGGGAACGT TTGCACCTTA TCGTGGGTAG
9001 AAAAAGAGGA AGTGGCGAAA AATGGTACTG ATCCGGTGGA AACCTATGAC CGTTTTACTC
9061 TGAAACTGGC GTTTCTGCGT CATGATGGCA GTTGGAGTGC CCCCTGGTCT TACGATATCA
9121 CAACGCAGGT GGAGGCGGTC ACTGACAAAA AACCTGACAC TGAACGGCTG GCGCTGGCCG
9181 CATCAGGCTT TCAGGGCGAG GATACTCTGC TGGTGTTTGT GTACAAAACC GGGGTGAGTT
9241 ACCCGGATTT TGGCGACAAC AATAAAAATG TGGCAGGCAT GACCATTTAC GGCGATGGCT
9301 CCTTCAAAAA GATGGAGAAC ACAGCACTCA GCGTTACAGC CAACTGAAAA ATACCTTTGA
9361 TATCATTCAT ACTCAAGGCA ACGACTTGGT AAGAAAGGCC AGCTATCGTT TCGCGCAGGA
9421 TTTTGAAGTG CCTGCCTCGT TGAATATGGG TTCTGCCATC GGTGATGATA GTCTGACGGT
9481 GATGGAAAAC GGGAATATTC CGCAGATAAC CAGTAAATAC TCCAGCGATA ACCTTGCTAT
9541 TACGCTACAT AACGCCGCTT TCACTGTCAG ATATGATGGC AGTGGCAATG TCATCAGAAA
9601 CAAACAAATC AGCGCCATGA AACTGACGGG GTTGGATGAA AGTCCCAGTA CGGCAATGCA
9661 TTTATCATCG CAAATACCGT TAAACATTAT GGCGGTTACT CTGATCTGGG GGGCCCGATC
9721 ACCGTTTTTA TTAAAACGGA AAAACTATAT TGCATCAGTT CAAGGCCACT TGATGAACGC
9781 AGATTACACT AGGCGTTTGA TTCTAACACC AGTTGAAAAT AATTATTATG CCAGATTGTT
9841 CGAGTTTCCA TTTTCTCCAA ACACAATTTT AAACACCGTT TTCACGGTTG GTAGCAATAA
9901 AACCAGTGAT TTTAAAAAGT GCAGTTATGC TGTTGATGGT AATAATTCTC AGGGCTTCCA
9961 GATATTTAGT TCCTATCAAT CATCCGGCTG GCTGGATATT GACACAGGTA TTAACAATAC
10021 TGATGTCAAA ATTACGGTGG TAGCTGGCAG TAAAACCCAC ACCTTTACGG CCAGTGACCA
10081 TATTGCTTCC TTGCCGGCAA ACAGTTTTGA TGCTATGCCG TACACCTTTA AGCCACTGGA
10141 AATCGATGCT TCATCGTTGG CCTTTACCAA TAATATTGCT CCTCTGGATA TCGTTTTTGA
10201 GACCAAAGCC AAAGACGGGC GAGTGCTGGG TAAGATCAAG CAAACATTAT CGGTGAAACG
10261 GGTAAATTAT AATCCGGAAG ATATTCTGTT TCTGCGTGAA ACTCATTCGG GTGCCCAATA
10321 TATGCAGCTC GGGGTGTATC GTATTCGTCT TAATACCCTG CTGGCTTCTC AACTGGTATC
10381 CAGAGCAAAC ACGGGCATTG ATACTATCCT GACAATGGAA ACCCAGCGGT TACCGGAACC
10441 TCCGTTGGGA GAAGGCTTCT TTGCCAACTT TGTTCTGCCT AAATATGACC CTGCTGAACA
10501 TGGCGATGAG CGGTGGTTTA AAATCCATAT CGGGAATGTT GGCGGTAACA CGGGAAGGCA
10561 GCCTTATTAC AGCGGAATGT TATCCGATAC GTCGGAAACC AGTATGACAC TGTTTGTCCC
10621 TTATGCCGAA GGGTATTACA TGCATGAAGG TGTCAGATTG GGGGTTGGAT ACCAGAAAAT
10681 TACCTATGAC AACACTTGGG AATCTGCTTT CTTTTATTTT GATGAGACAA AACAGCAATT
10741 TGTATTAATT AACGATGCTG ATCATGATTC AGGAATGACG CAACAGGGGA TCGTGAAAAA
10801 TATCAAGAAA TACAAAGGAT TTTTGAATGT TTCTATCGCA ACGGGCTATT CCGCCCCGAT
10861 GGATTTCAAT AGTGCCAGCG CCCTCTATTA CTGGGAATGT TCTATTACAC CCCGATGATG
10921 TGCTTCCAGC GTTTGCTACA GGAAAAACAA TTCGACGAAG CCACACAATG GATAAACTAC
10981 GTCTATAATC CCGCCGGCTA TATCGTTAAC GGAGAAATCG CCCCCTGGAT CTGGAACTGC
11041 CGGCCGCTGG AAGAGACACT CCTGGAATGC CAATCCGTTG GATGCCATTG ATCCGGATGC
11101 CGTCGCACAA TATGACCCGA CACACTATAA AGTTGCCACC TTTATGCGCC TGTTGGATCA
11161 ACTTATTCTG CGCGGCGATA TGGCCTATCG CGAACTGACC CGCGATGCGT TGAATGAAGC

Fig.2.

11221	CAAGATGTGG	TATGTGCGTG	CTTTGGAATT	GCTGGGTGAT	GAGCCGGAGG	ATTACGGCAG
11281	CCAACAGTGG	GCCGCACCGT	CTCTTTCCGT	GGCGGGCAAC	CACACTGTGC	AAGCGGGCTA
11341	TCAACAAGAC	CTTACGGCGC	TAGACAACGG	AGAAGGTTGC	ACTCAACCCC	GCAACGCTAA
11401	CTCGTTGGTG	GTTTGGTCCT	GCCGGAATAT	AACCCGGAAT	CAACCGATTA	CTGGCAAACC
11461	TGCGTTTGCG	CCTGGTTAAC	CTGCGCCATA	ATCCTTCCAT	GACGGGCAAC	CGTTATCGCT
11521	GGCGAATTAC	GCGAGCCTAC	GATCCGAAAG	CGCTGCTCAC	CAGTATGGTA	CAGCCTTCTC
11581	AGGGCGGTAG	TGCAGTGCTG	CCCGGCACAT	TGTCGTTATA	CCGCTTCCCG	GTGATGCTGG
11641	AGCGGGCCCG	CAATCTGGTA	GCGCAATTAA	CCCAGTTCGG	CACCTCTCTG	CTCAGTATGG
11701	CAGAGCATGA	TGATGCCGAT	GAACTCACCA	CGTTGCTACT	ACAGCAGGGT	ATGGAACTGG
11761	CGACACAGAG	CATCCGTATT	CAGCAACGAA	CTGTCGATGA	AGTGGATGCT	GATATTGCTG
11821	TATTGGCAGA	GAGCCGCCGC	AGTGCACAAA	ATCGTCTGGA	AAAATACCAG	CAGCTGTATG
11881	ACGAGGATAT	CAACCACGGA	GAACAGCGTG	CGATGTCACT	GTTTGATGCG	GCGGCAGGTC
11941	AGTCTCTGGC	CGGGCAGGCG	CTCTCAGTAG	CAGAAGGGGT	GGCTGACTTA	GTTCCAAACG
12001	TGTTCGGTTT	CGCTTGTGGC	GGCAGTCGTT	GGGGGGCAGC	ACTGCGTGCT	TCCGCCTCCG
12061	TGATGTCGCT	TTCTGCCACA	GCTTCCCAAT	ATTCCGCAGA	CAAAATCAGC	CGTTCGGAAG
12121	CCTACCGCCG	CCGCCGTCAG	GAGTGGGAAA	TTCAGCGTGA	TAATGCTGAC	GGTGAAGTCA
12181	AACAAATGGA	TGCCCAGCTG	GAAAGCCTGA	AAATACGCGG	CGAAGCAGCA	CAGATGCAGG
12241	TGGAATATCA	GGAGACCCAG	CAGGCCCATA	CTCAGGCTCA	GTTAGAGCTG	TTACAGCGTA
12301	AATTCACAAA	CAAAGCGCTT	TACAGTTGGA	TGCGCGGCAA	GCTGAGTGCT	ATCTATTACC
12361	AGTTCTTTGA	CCTGACCCAG	TCCTTCTGCC	TGATGGCACA	GGAAGCGCTG	CGCCGCGAGC
12421	TGACCGACAA	CGGTGTTACC	TTTATCCGGG	GTGGGGCCTG	GAACGGTACG	ACTGCGGGTT
12481	TGATGGCGGG	TGAAACGTTG	CTGCTGAATC	TGGCAGAAAT	GGAAAAAGTC	TGGCTGGAGC
12541	GTGATGAGCG	GGCACTGGAA	GTGACCCGTA	CCGTCTCGTT	GGCACAGTTC	TATCAGGCCT
12601	TATCATCAGA	CAACTTTAAT	CTGACCGAAA	AACTCACGCA	ATTCCTGCGT	GAAGGGAAAG
12661	GCAACGTAGG	AGCTTCCGGC	AATGAATTAA	AACTCAGTAA	CCGCCAGATA	GAAGCCTCAG
12721	TGCGATTGTC	TGATTTGAAA	ATTTTCAGCG	ATACCCCGGA	AAGCTTTGGC	AATACCCGTC
12781	AGTTGAAACA	AGTGAGTGTC	ACCTTGCCGG	CGCTGGTTGG	TCCGTATGAA	GATATCCGGG
12841	CGGTGCTGAA	TTACGGCGGC	AGCATCGTCA	TGCCACGCGG	TTGCAGTGCT	ATTGCTCTCT
12901	CCCACGGCGT	GAATGACAGT	GGTCAATTTA	TGCTGGATTT	CAACGATTCC	CGTTATCTGC
12961	CGTTTGAAGG	TATTTCCGTG	AATGACAGCG	GTAGCCTGAC	GTTGAGTTTC	CCGGATGCGA
13021	CTGATCGACA	GAAAGCGCTG	CTGGAGAGCC	TGAGCGATAT	CATTCTGCAT	ATCCGCTATA
13081	CCATTCGTTC	TTAATTAAAA	CATTGTGATA	GGCAGGCTCC	TGAGGGAGCC	TGTTTAAGGA
13141	GTTTTTATGC	AGGGTTCAAC	ACCTTTGAAA	CTTGAAATAC	CGTCATTGCC	CTCTGGGGGC
13201	GGATCACTAA	AAGGAATGGG	AGAAGCACTC	AATGCCGTCG	GAGCGGAAGG	GGAGCGTCAT
13261	TTTCACTGCC	CTTGCCGATC	TCTGTCCGGC	GTGGTCTGGT	GCCGGTGCTA	TCACTGAATT
13321	ACAGCAGTAC	TGCTGGCAAT	GGGTCATTCG	GGATGGGGTG	GCAATGTGGG	GTTGGTTTTA
13381	TCAGCCTGCG	TACCGCCAAG	GGCGTTCCGC	ACTATACGGG	ACAAGATGAG	TATCTCGGGC
13441	CGGATGGGGA	AGTGTTGAGT	ATTGTGCCGG	ACAGCCAAGG	GCAACCAGAG	CAACGCACCG
13501	CAACCTCACT	GTTGGGGACG	GTTCTGACAC	AGCCGCCTAC	TGTTACCCGC	TATCAGTCCC
13561	GCGTGGCAGA	AAAAATCGTT	CGTTTAGAAC	ACTGGCAGCC	ACAGCAGAGA	CGTGAGGAAG
13621	AGACGTCTTT	TTGGGTACTT	TTTACTGCGG	ATGGTTTAGT	GCACCTATTC	GGTAAGCATC
13681	ATCATGCACG	TATTGCTGAC	CCGCAGGATG	AAACCAGAAT	TGCCCGCTGG	CTGATGGAGG
13741	AAACCGTCAC	GCATACCGGG	GAACATATTT	ACTATCACTA	TCGGGCAGAA	GACGATCTTG
13801	ACTGTGATGA	GCATGAACTT	GCTCAGCATT	CAGGTGTTAC	GGCCCACCGT	TATCCTGGCA
13861	AGTCCACTAT	GGCAATACTC	AGCCGGAAAC	CGCTTTTTTC	GCGGTAAAAT	CAGGTATCCC
13921	TGTTGATAAT	GACTGGTTGT	TTCATCTGGT	ATTTGATTAC	GGTGAGCGCT	TATCTTCGCT
13981	GAACTCCGTA	CCCGAATTCA	ATGTGTCAGA	AAACAATGTG	TCTGAAAACA	ATGTGTCTGA
14041	AAAATGGCGT	TGTCGTCCGG	ACAGTTTCTC	CCGCTATGAA	TATGGGTTTG	AAATTCGAAC
14101	CCGTCGCTTG	TGTCGCCAAG	TTCTGATGTT	TCATCAGCTG	AAAGCGCTGG	CAGGGGAAAA
14161	GGTTGCAGAA	GAAACACCGG	CGCTGGTTTC	CCGTCTTATT	CTGGATTATG	ACCTGAACAA
14221	CAAGGTTTCC	TTGCTGCAAA	CGGCCCGCAG	ACTGGCCCAT	GAAACGGACG	GTACGCCAGT
14281	GATGATGTCC	CCGCTGGAAA	TGGATTATCA	ACGTGTTAAT	CATGGCGTGA	ATCTGAACTG
14341	GCAGTCCATG	CCGCAGTTAG	AAAAAATGAA	CACGTTGCAG	CCATACCAAT	TGGTTGATTT
14401	ATATGGAGAA	GGAATTTCCG	GCGTTACTTT	ATCAGGATAC	TCAGAAAGCC	TGGTGGTACC
14461	GTGCTCCGGT	ACGGGATATC	ACTGCCGAAG	GAACGAATGC	GGTTACCTAT	GAGGAGGCGA
14521	AACCACTGCC	ACATATTCCG	GCACAACAGG	AAAGCGCGAT	GTTGTTGGAC	ATCAATGGTG
14581	ACGGGCGTCT	GGATTGGGTG	ATTACGGCAT	CAGGGTTACG	GGGCTACCAC	ACCATGTCAC
14641	CGGAAGGTGA	ATGGACACCC	TTTATTCCAT	TATCCGCTGT	GCCAATGGAA	TATTTCCATC
14701	CGCAGGCAAA	ACTGGCTGAT	ATTGATGGGG	CTGGGCTGCC	TGACTTAGCG	CTTATCGGGC
14761	CAAATAGTGT	ACGTGTCTGG	TCAAATAATC	CGGCAGGATG	GGATCGCGCT	CAGGATGTTA
14821	TTCATTTGTC	AAATAAGCCA	CTGCCGGTTC	CCGGCAAAAA	TAAGCGTCAT	CTTGTCGCAT
14881	TCAGTGATAT	GACAGGCTCC	GGGCAATCAC	ATCTGGTGGA	AGTTACGGCA	AATAGCGTGC
14941	GCTACTGGCC	GAACCTGGGG	CATGGAAAAT	TTGGTGAGCC	TCTGATGATA	ACAGGCTTCC
15001	AAATTACGGG	GAAACGTTTA	ACCCCCACAG	ACTGTATATG	GTAGACCTAA	ATGGCTCAGG

Fig.2.

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15061  CACCACCCGA TTTTATTTAT GCCCGCAATA CTTACCTTGA ACTCTATGCC AATGAAAGCG
15121  GCAATCATTC TGCTGAACCT CAGCGTATTG ATCTGCCGGA TGGGGTACGT TTTGATGATA
15181  CTTGTCGGTT ACAAATAGCG GATACACAAG GATTAGGGAC TGCCAGCATT ATTTTGACGA
15241  TCCCCCATAT GAAGGTGCAG CACTGGCGAT TGGATATGAC CATATTCAAG CCTTGGCTGC
15301  TGAATGCCGT CAATAACAAT ATGGGAACAG AAACCACGCT GTATTATCGC AGCTCTGCCC
15361  AGTTCTGGCT GGATGAGAAA TTACAGGCTT CTGAATCCGG GATGACGGTG GTCAGCTACT
15421  TACCGTTCCC GGTGCATGTG TTGTGGCGCA CGGAAGTGCT GGATGAAATT TCCGGTAACC
15481  GATTGACCAG CCATTATCAT TACTCACATG GTGCCTGGGA TGGTCTGGAA CGGGAGTTTC
15541  GTGGTTTTGG GCGGGTGACG CAAACTGATA TTGATTCACG GGCGAGTGCG ACACAGGGGA
15601  CACATGCTGA ACCACCGGCA CCTTCGCGCA CGGTTAATTG GTACGGCACT GGCGTACGGG
15661  AAGTCGATAT TCTTCTGCCC ACGGAATATT GGCAGGGGGA TCAACAGGCA TTTCCCCATT
15721  TTACCCCACG CTTTACCCGT TATGACGAAA AATCCGGTGG TGATATGACG GTCACGCCGA
15781  GCGAACAGGA AGAATACTGG TTACATCGAG CCTTAAAAGG ACAACGTTTA CGCAGTGAGC
15841  TGTATGGGGA TGATGATTCT ATACTGGCCG GTACGCCTTA TTCAGTGGAT GAATCCCGCA
15901  CCCAAGTACG TTTGTTACCG GTGATGGTAT CGGACGTGCC TGCGGTACTG GTTTCGGTGG
15961  CCGAATCCCG CCAATACCGA TATGAAGGGG TTGTTACCGA TTCCACAGTG CAGCCAAAAG
16021  ATTGTCCTTA AATATGATGC GTTAGGATTT CCGCAGGACA ATCTTGAGAT TGCCTATTCG
16081  AGACGTCCAC AGCCTGAGTT CTCGCCTTAT CCGGATACCC TGCCCGAAAC ACTTTTCACC
16141  AGCAGTTTCG ACGAACAGCA GATGTTCCTT CGTCTGACAC GCCAGCGTTT TTCTTATCAC
16201  CATCTGAATC ATGATGATAA TACGTGGATC ACAGGGCTTA TGGATACCTC ACGCAGTGAC
16261  GCACGTATTT ATCAAGCCGA TAAAGTGCCG GACGGTGGAT TTTCCCTTGA ATGGTTTTCT
16321  GCCACAGGTG CAGGAGCATT GTTGTTGCCT GATGCCGCAG CCGATTATCT GGGACATCAG
16381  CGTGTAGCAT ATACCGGTCC AGAAGAGCAA CCCGCTATTC CTCCGCTGGT GGCATACATT
16441  GAAACCGCAG AGTTTGATGA ACGATCGTTG GCGGCTTTTG AGGAGGTGAT GGATGAGCAG
16501  GAGCTGACAA AACAGCTGAA TGATGCGGGC TGGAATACGG CAAAAGTGCC GTTCAGTGAA
16561  AAGACAGATT TCCATGTCTG GGTGGGACAA AAGGAATTTA CAGAATATGC CGGTGCAGAC
16621  GGATTCTATC GGCCATTGGT GCAACGGGAA ACCAAGCTTA CAGGTCAAAC GACAGTGACG
16681  TGGGATAGCC ATTACTGTGT TATCACCGCA ACAGAGGATG CGGCTGGCCT GCGTATGCAA
16741  GCGCATTACG ATTATCGATT TATGGTTGCG GATAACACCA CAGATATCAA TGATAACTAT
16801  CACACCGTGA CGTTTGATGC ACTGGGGACG GTAACCAGCT TCCGTTTCTG GGGGACTGAA
16861  AACGGTGAAA AACAAGGATA TACCCCTGCG GAAAATGAAA CTGTCCCCTT TATTGTCCCC
16921  ACAACGGTGG ATGATGCTCT GGCATTGAAA CCCGGCATAC CTGTTGCAGG GCTGATGGTT
16981  TATGCCCCTC TGAGCTGGAT GGTTCAGGCC AGCTTTTCTA ATGATGGGGA GCTTTATGGA
17041  GAGCTGAAAC CGGCTGGGAT CATCACTGAA GATGGTTATC TCCTGTCGCT TGCTTTTCGC
17101  CGCTGGCATC AAAATAACCC TGCCGCTGCC ATGCCAAAGC AAGTCAATTC ACAGAACCCA
17161  CCCCATGTAC TGAGTGTGAT CACCGACCGC TATGATGCCG ATCCGGAACA ACAATTACGT
17221  CAAACGTTTA CGTTTAGTGA TGGTTTTGGG CGAAACCTTA CAAACAGCCG TACGCCATGA
17281  AAGTGGTGAA GCCTGGGTAC CTGATGAGTA TGGAGCCAAT GTGGCTGAAA ATCAAGGCGC
17341  CCCTGAAACG GGCGATTACA AATTTCCCGT TGGGCAATTT CCCGGACGTA CAGAATATTA
17401  ACGGGAAAAG GCAAAGCCCC TGCGTTACGT TTCAAACCGT ATTCCTGAAA TAATTTGGGC
17461  AACTATGTCA AGTTGACCAA AAAATGCCCG GCAGGATATG TATGCCGATA CCCATTACTA
17521  TGATCCGTTG GGGCGTGAAT ATCAGGTTAT CACGCCAAAG GCGGGTTGCG TCGATCCTTA
17581  TTCACTCCCT GGTTTGTGGT GAATGAAGTT GAAAATGACA CTCCCGGTGA ATGACAGCAT
17641  AAAGCTCAGT GATGCCTGTT CACTGAACAG ACATCACTCC ATTTAGGAAT GAATCATGAA
17701  GAATTTCGTT CACAGCAATA CGCCATCCGT CACCGTACTG GACAACCGTG GTCAGACAGT
17761  ACGCGAAATA GCCTGGTATC GGCACCCCGA TACACCTCAG GTAACCGATG AACGCATCAC
17821  CGGTTATCAA TATGATGCTC AAGGATCTCT GACTCAGAGT ATTGATCCGC GATTTTATGA
17881  ACGCCAGCAG ACAGCGAGTG ACAAGAACGC CATTACACCC AATCTTATTC TCTTGTCATC
17941  ACTCAGTAAG AAGGCATTGC GTACGCAAAG TGTGGATGCC GGAACCCGTG TCGCCCTGCA
18001  TGATGTTGCC GGGCGTCCCG TTTTAGCTGT CAGCGCCAAT GGCGTTAGCC GAACGTTTCA
18061  GTATGAAAGT GATAACCTTC CGGGACGATT GCTAACGATT ACCGAGCAGG TAAAAGGAGA
18121  GAACGCCTGT ATCACGGAGC GATTGATTTG GTCAGGAAAT ACGCCGGCAG AAAAAGGCAA
18181  TAATTTGGCC GGCCAGTGCG TGGTCCATTA TGATCCCACC GGAATGAATC AAACCAACAG
18241  CATATTGTTA ACCAGCATAC CCTTGTCCAT CACACAGCAA TTAGTGAAAG ATGACAGCGA
18301  AGCCGATTGG CACGGTATGG ATGAATTTGG CTGGAAAAAC GCGCTGGCGC CGGAAAGCTT
18361  CACTTCTGTC AGCACAACGG ATGCTACCGG CACGGTATTA ACGAGTACAG ATGCTGCCGG
18421  AAACAAGCAA CGTATCGCCT ATGATGTGGC CGGTCTGCTT CAAGGCAGTT GGTTGGCGCT
18481  GAAGGGGAAA CAAGAACAAG TTATCGTGAA ATCCCTGACC TATTCGGCTG CCAGCCAGAA
18541  GCTACGGGAG GAACATGGTA ACGGGATAGT GACTACATAT ACCTATGAAC CCGAGACGCA
18601  ACGAGTTATT GGCATAAAAA CAGAACGTCC TTCCGGTCAT GCCGCTGGGG AGAAAATTTT
18661  ACAAAACCTG CGTTATGAAT ATGATCCTGT CGGAAATGTG CTGAAATCAA CTAATGATGC
18721  TGAAATTACC CGCTTTTGGC GCAACCAGAA AATTGTACCG GAAAATACTT ACACCTATGA
18781  CAGCCTGTAC CAGCTGGTTT CCGTCACTGG GCGTGAAATG GCGAATATTG GCCGACAAAA
18841  AAACCAGTTA CCCATCCCCG CTCTGATTGA TAACAATACT TATACGAATT ACTCTCGCAC
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Fig.2.

18901	TTACGACTAT	GATCGTGGGG	GAATCTGACC	AGAATCGCAT	AATTCACGAT	CACCGGTAAT
18961	AACTATACAA	CGAACATGAC	CGTTTCAGAT	CACAGCAACC	GGGCTGTACT	GGAAGAGCTG
19021	GCGCAAGATC	CCACTCAGGT	GGATATGTTG	TTCACCCCCG	GCGGGCATCA	GACCCGGCTT
19081	GTTCCCGGTC	AGGATCTTTT	CTGGACACCC	CGTGACGAAT	TGCAACAAGT	GATATTGGTC
19141	AATAGGGAAA	ATACGACGCC	TGATCAGGAA	TTCTACCGTT	ATGATGCAGA	CAGTCAGCGT
19201	GTCATTAAGA	CTCATATTCA	GAAGACAGGT	AACAGTGAGC	AAATACAGCG	AACATTATAT
19261	TTGCCAGAGC	TGGAATGGCG	CACGACATAT	AGCGGCAATA	CATTAAAAGA	GTTTTTGCAG
19321	GTCATCACTG	TCGGTGAAGC	GGGTCAGGCA	CAAGTGCGGG	TGCTGCATTG	GGAAACAGGC
19381	AAACCGGCGG	ATATCAGCAA	TGATCAGCTG	CGCTACAGTT	ATGGCAACCT	GATTGGCAGT
19441	AGCGGGCTGG	AATTGGGACA	GTGACGGGCA	GATCATTAGT	CAGGAAGAAT	ATTACCCCTA
19501	TGGGGGAACC	GCCGTGTGGG	CACCCGAAAT	CAGTCAGAAG	CTGATTACAC	AAGCCGGCGT
19561	TATTCTGGCA	AAGAGCGGGA	TGCAACAGGG	TTGTATTACT	ACGGCTATCG	TTATTATCAA
19621	TCGTGGACAG	GGCGATGGTT	GAGTGTAGAT	CCTGCCGGTG	AGGCCGATGG	TCTCAATTTG
19681	TTCCGAATGT	GCAGGAATAA	CCCCATCGTT	TTTTCTGATT	CTGATGGTCG	TTTCCCCGGT
19741	CAGGGTGTCC	TTGCCTGGAT	AGGGAAAAAA	GCGTATCGAA	AGGCAGTCAA	CATCACGACA
19801	GAACACCTGC	TTGAACAAGG	CGCTTCCTTT	GATACGTTCT	TGAAATTAAA	CCGAGGATTG
19861	CGAACGTTTG	TTTTGGGTGT	GGGGGTACAA	GTCTGGGGGT	GAAGCGGCCA	CGATTGCAGG
19921	AGCGTCGCCT	TGGGGGATCG	TCGGGGCTGC	CATTGGTGGT	TTTGTCTCCG	GGGCGGTGAT
19981	GGGGTTTTTC	GCGAACAACA	TCTCAGAAAA	AATTGGGGAA	GTTTTAAGTT	ATCTGACGCG
20041	TAAACGTTCT	GCTCCTGTTC	AGGTAGGCGC	TTTTGTTGTC	ACATCGCTTG	TGACGTCTGC
20101	ACTATTTAAC	AGCTCTTCGA	CAGGTACCGC	CATTTCCGCA	GCAACAGCGG	TCACCGTTGG
20161	AGGATTAATG	GCTTTAGCCG	GAGAACATAA	CACGGGCATG	GCTATCAGTA	TTGCCACACC
20221	CGCCGGACAA	AGTACGCTGG	ATACGCTCAG	GCCCGGTAAT	GTCAGCGCGC	CAGAGCGGTT
20281	AGGGCACTAT	CAGGCGCAAT	TATTGGCGGC	ATATTACTTG	GCCGCCATCA	GGGAAGTTCT
20341	GAGCTGGGTG	AACGGGCAGC	GATTGGTGCT	ATGTATGGTG	CTCGATGGGG	AAGGATCATT
20401	GGTAATCTAT	GGGATGGCCC	TTATCGGTTT	ATCGGCAGGT	TACTGCTCAG	AAGAGGCATT
20461	AGCTCTGCCA	TTTCCCACGC	TGTCAGTTCC	AGGAGCTGGT	TTGGCCGAAT	GATAGGAGAA
20521	AGTGTCGGGA	GAAATATTTC	TGAAGTATTA	TTACCTTATA	GCCGTACACC	CGGTGAATGG
20581	GTTGGTGCAG	CCATTGGCGG	GACAGCCGCG	GCCGCTCATC	ATGCCGTTGG	AGGGGAAGTT
20641	GCCAATGCCG	CTAGCCGGGT	TACCTGGAGC	GGCTTTAAGC	GGGCTTTTAA	TAACTTCTTC
20701	TTTAACGCCT	CTGCACGTCA	TAATGAATCC	GAAGCATAAC	AATCATGTTC	ATTCCCACTT
20761	TGTCATGGAT	GACAAGGTGG	GTTTTTCGGA	TGTGTGGACA	GAGACCCGTA	CAGGGTCTCT
20821	GTCCAGTTAA	TTTTTGGATC	AAGAACGAAT	GGTGTAACGG	ATATGCAAAA	TGATATCGCT
20881	CAGGCTGAGC	AATAAGCTTT	TCTGTTTACC	ACTGATACCG	GGAAAACTGA	GGGTTAATGT
20941	GCCTGTATCG	GCCACAGGAA	GCCCTTCAAA	TGGCAGGTAC	TTAGCATCAT	TGAAATCCAT
21001	CTGGAATTGA	CCACTGTCAT	TCATGCCATG	TGAGATCACA	ATCGCTTTGC	AGCCACGTGG
21061	CATCATTGTA	CTGCCGCCAT	AACTCAGTAT	TGCCCGGACA	TCCTGATAAG	GCCCTAAAAG
21121	GGCAGGTAAC	GTCACACTGA	TTTGTTTGAT	ACGGCGTGTA	TTACCTAAAC	CGTCAGGATA
21181	ATCGGTAGCA	ATATTCAGAT	CCGATAATTT	GAGGCTGGCT	TGCAGTTGTG	TCCCTTCGAC
21241	GTTCAAACCG	TTAAGCGTTG	TGCCTGCACT	GCCTTCACCT	GCATTGACTA	ACTCAGTCAC
21301	TTTATCTTTT	AAAATGAAAC	TATTTTCTGT	CAGACCAGCA	TACACTTCAG	CCAGAGAAAC
21361	GGTTCTGGTG	ACCTCCAGTG	CCCGTTCATC	TTTTTCCAAA	TAGCTTTTTT	CCATCTGTGC
21421	TAAATTCAGC	ATCAGGGTTT	CACCCGCTAA	TAAACCCGCA	TAAGTCCCAT	GCCAAGCACC
21481	TGGTTTAATA	AAGTGTGCTG	CCGCATTATT	CAATTCATAC	TGATAAGTTT	GCTCTGCCAT
21541	TAAACAGAGT	GAGACCGCCA	AATCATAAAA	CTGATAATAA	ATAGCGGACA	ACGTTCCACG
21601	GAGCCAGTTG	TATAGCGCTG	CATTACTGAA	TTTACTTTGC	AGAAAGGCTA	ACTGCGCCTG
21661	AGTTTGTGCC	TGCTGAGTTT	CCAGATAGTT	TTTTTGTAAT	ACTGCCGCTT	CACGACGTAC
21721	AGCCAGCGTC	GCTAATTGAG	CATCAATTTG	TTTTATCTCA	GCTTCCGCAT	TATTGCGCTG
21781	AATTTCCCAC	TCTTGCCGAC	GGCGACGGTA	TATTTCTGAT	TGGCTGATTT	TGTCTGCGGC
21841	AATACGTGTT	GCTGACGCAG	AAATTTCGAT	ACCAATCGCA	CTGGCATTGA	AAAGCGCCCC
21901	AAAACGGGAA	CCTCCCACAG	CAAAACCGTA	AATATTGGGG	ACGAGATCTG	CCGCGGCGGC
21961	GGCCATATGC	AGGGCTGTGC	CGCTGGTGCT	CAAGACCGAT	GAAGAGAGGT	AAAGATCCAT
22021	CGCTTGTTTT	TCACCAGCGT	TAACATCTTC	GTCGTACAGC	GTATTGAAAC	TGTCAAAACG
22081	AGACTGTGCA	CCATGACGGC	TTTCTTGAAG	CGCCAATTTA	TCAGCATCAA	TTTCAGCCAT
22141	GACCTTATCC	TGCATTTTAA	TACTTTGCAG	GGCTAACTCA	CTGCCTTGAG	TTTGCAGTAT
22201	TTCAGCCAAG	GCTTCTGCAT	CCTGCCGTTC	AGTAATGCTG	AGCAGGGTAT	TGCCAAATTG
22261	TATCAACTGG	CTTACCCCCC	ACTTGGCATT	TTCCAGAATC	ACCGGAAAAC	GGTACATCGG
22321	CATCACTGCA	TGAGGTAAAT	CGCCGCCGCC	TTGTGAAGCA	GTGATGGCAG	CACTGAGTAA
22381	CATGGACGGA	TCTGCGGGCG	TGGCATAGAG	AGATAATGAC	AGTGGCTGAC	CGTCGATTGT
22441	CAGGTTATGG	CGTAAGTTAT	AGAGGCGTTG	CGTCAATGTC	TGCCAGTAAC	CTTGCAGTTT
22501	TTTATTAATT	TGAGGGAGGA	ACAATGCGGT	TAACGAAATT	TGCCGTACGT	TTCGTGGGTA
22561	ATGCAGCGCG	CTGACGCAGT	TGCAGCATTT	TATGTTGATA	ATGATGCCGC	ATTGTTTGGC
22621	TGGCAGCTTC	TTCCAGCCGT	GGCTCTGACC	AATCGTTATC	CAATGAAAAA	TAAGGCTCAT
22681	CACCCAATAA	AGTGAGCGCC	TGTACATACC	ACATTTTAGC	TTCGTTTAAG	GTATCACGTT

Fig.2.

22741 CAAGCTGGCG ATAGGCGCTA TCTCCGCGGG TAATCAACAA ATCCAGCATT TTCATAAAGG
22801 TAGCCACTTT ATAGTGCATC GGATCATGCT GGGCAACGGC GTCCGGATCG ACCGAATCCA
22861 GCGGATTGGC ATTCCAGGAC GTATCTTCCT CCAATGGGCG GACGTTCCAG TAATAATCCT
22921 GCATTTCACC CTGAACCGAA TATCCGGTCG GGTTCAGATA TAGCGCAGCC AGCGTGTCGA
22981 TCCGGTAAAA TCTGCTCTTG CAATAAGCGC TGGAATACCA TCATGGGCGT TGTAATAGAA
23041 CAATCCCAAG AAATAGATTG CATTGGCGCC GTTTGAAATC CATGGGTTCA GTGTTATTTT
23101 TCATGACACG ACTTGAATAC CCCTTTTATA TTTTTTGATA TTTTTTACTA TCCCCTGTTG
23161 TGTCATTCCC GAATCATGAT CGGCATCATT AGTGAATATA AATTGATTTT TCGTCTCATC
23221 AAAATAAAAG AAAGCAGATT CCCAGGATTT GTCATAGATA ATTTTTTTGT ACCCAACCCC
23281 TAATCTGACA CCTTCACGTA TGTAATATCC TTTAGCATAG GGAACAAAGA GCGTTACTGT
23341 GGTTTCAATA TCAGATAACA TTCCTTCGTA ATAAGGTTGT CTGGCAGAAT TGCCATCAAT
23401 ATTCCCAATA TGGATCTTAA ACCAACGTTC ATCACCATGC TCCTCTTTAT TGTAGGGGGG
23461 CAACTTAAAT GTCGCATAAA ACCCTTCACC TAATTGCGGC TCTGGTAAAT TTTGCGTTTC
23521 CATACTTAAA ACATTATCAA TACCAATATT GGCTCTTTCA GCTAATTTTC TGGAAAATAA
23581 AGTATTTAAC CGGGTTCTGT AAGGGCCAAT CTGCATATAT TGTGTGCCTG ATGGCATTTT
23641 ATGCAGTGAT ATAACGTTAC TTGTATCTTT GGATTTTAGT TTTATATGAA TTGGCGATTC
23701 AATAACAATA TCGTTATAAC CGCCGTCGGG TTGCTTAATA ATAAACTCGC TCACCAGAGG
23761 AATATCATAG CCTTCAATAT CAACTTTTAC TTGATTAAAA TCATATACCA TAGGGTCAGA
23821 TTCGTGTGAA GGTTTAGATG CCACATGGTC TTCAGCATTT AACTCCACTA GAATATCAGA
23881 GCCATTTTTT AATAAAAAAC TAATGTTTTT ATCTTGGATC TGTTCGATCA TAGATGAAGC
23941 AAGTTTTATT ATCTGTGGCT GGTTGAACAT AAATACACCC ATGGATCCTC GCGAAGGAAC
24001 AGTGCCGCAA TATTTCCCAT GTTATTAATG ATTGAAACAT CATTAGTAAA TGATTCACAT
24061 ATAGTATGCC ATACTCCTGT GTTATCTTTC CAATCTAATA CTATGTTAGT ATCAAGTTTG
24121 AATTCAGCAT CATCTGATTC ATAATCATAA TTTATACCAA CTCCAATTTC TGATTTTCTA
24181 GGAATTTTTT CCTTGGTTCT TAGATGCATT AACACTCTAA AATATTCGGC ATTTTTAAGA
24241 TCGATGGAAA TAATAAAATC CAAAGTTCCA TAATGAAAAA CTTCTTCTTC TTTTCCAAGC
24301 ATTTCATCAT GTCTATCATA ATCAAATAAA ATAACCGTTT CATCTTCTAC CATCGATAAC
24361 AGGTATTTAA CCTCATCATT ATATATATTG CCTTTTGAAA AATTAATTTC CATTGAAGGA
24421 TTGAACGTTA AATTAATATG ACCATTTCCT GGTGATATAT ACGAGAGATC AAAAATATTT
24481 CCGGTAAAAC TGGCTAATTT ATTTTTTGTG GTTATAGATT CCTTATATTC GGCCAAATAA
24541 TCTGTAGCAA ATTGATTGTT GACTTTGTAT TCTGTCCTGG TATCAAGTTC TGATAATGTG
24601 CTCTTAACAA TGGCGTCTAA ATCATTTTCT GTGAGAATGG ATAATGTCAT ATCAGGGTTA
24661 ATGGTCATCC CTTCTCTTGC AGGAAGACTA TTAAAAGAAT AATTGTCTTT TTTCTCATGG
24721 AAATAAACAA TAATGACGTC TTTTTCATAA TCAGAAGAAC AATACATACC AATGCTGGCT
24781 TTTTTATTGA TCAGGTTTTC TATTTTATCA GTCACATTAA AATTAAACGG TGAGCTCCAG
24841 CTGCCATCAT AACGAATATG TGACAGTTTT AATATATAAT CAGTGATATC TATCTTGCCA
24901 TCTTCACTTT CATTTTTCAG CTCTTTTTGT TCCAGCCACA GTAAATACAA ACGAGACTTG
24961 TAAATAACAG GTCTGATATT TTCCTGCCAT ACATTGATGG GTATTTCAAT TTTTTTCCAT
25021 TCTCCCCAGG CATTGGCAGC AAATTGACCG TGCTGGCACT TTTGGTGATC GACATTGCGC
25081 CAATAATATA TTCTGGGTTC TGTCTGGCTA TAACCAATTA AATAAGTGAG CCCCTCATTG
25141 ACATTAATAC TGTCATGATA TCCGCTAATC ACCTGCAAGT TAGCGACATC TTCAAATGCG
25201 GTCAGATAAT TTTTAAAGCT ATCTTCAACG GTATCGATAT TTAACTGACT TTGGGAAAGT
25261 TGCTGTAACA GGTTGTTCAT CATACCTGTC TGACCAATAC GAATCGTGGG GTCGATATAG
25321 TTTTCCGGAT AATAGGCCAG TTCAGATACG CCGGCCCAGG TGCTATACCG TCGATTGTAG
25381 GTTTCCCAGT CGCAGAAGAA CTGACGGGTT TTCACTGGCT TTGATACTTT TCCTTCAACA
25441 TTATTCAACG CCCGGTTGAC ATATAACTGA ATGCTGGCAA TGGCTTCTGC CACACGGGTG
25501 GTTTTCACTT GGGCAGAAAC TTGGTTATCA ATCAGCAGAT AGCTGTACAA CTCATCCCGG
25561 CTCTTAATCT GTTGAGGTGC ACCATTTTTG ATGTAGTAAG CACTGGCCGC TGTCGTCGTG
25621 GCTTCATCCA GCCATGCCTG AAGCTGGTCG GATTGTTGAC TGTTCAGTCC CGCCTGCAAC
25681 AAAGTACTGG CGGCTTGCCA ATCATCAAAT GTTGGCATCG GGGTTTCCGG TTCACCGACA
25741 TATTTTAATT TTATGAGTGC AGCAACACCA TCCGGGGTAA TACCCAATGT AGCAGCGACA
25801 TCCAGCCATT GCAGAGTGAC ATCTATAAGT TCTCCAGTTG GTAAAGGTAT TCACTCCCAA
25861 ACCGGTCTGT TGCAATGCTT GTGTCACAAC CTGAGCATCA AAATTTTAAC GCCACCGCCA
25921 AATTGTTCGG CAGTCAACGC TCCTAAGTTC CAAATGCTGT TAAGATTCTG TCGCGTAGCT
25981 TCACAACGCA TGATCACAGC ATGGAAGCGG GTCAGCGCTT GCAAAGTGGG GAGATCATGT
26041 TGCAGTGCTG TGGTTTCTGA TTGGAATTTC TCCGGTTTTG TCACCAACAG GGTCAGTTCG
26101 TTTTCGCTGA GTCCAATATT GCGCACAATC AGAGAAAGTT GCCCCAGTAC CTGACAAAAA
26161 GCCACCATGT TGCTGGTTTC ATTCTCTGAG CGATCACGGT TAGCCGCAAT AATCATGAAA
26221 TCATCGAATG TCAGTCCTTG TGGTTTTATC TGATTAATCC ACAGCAAAAT AGTTTCTGCT
26281 GTTTTGGCTG AATCCATTTG AATGCTGGCA GCAATCAGCG GGGCAGCTGC ACGGATCAGT
26341 TCGTCATCAC CGAGTGAAAG TGTTGATAAT CCATTACTTA GTGTCGTGAT AAGGTTTTCA
26401 ATATCCGGCG TAAGGACAGT GCTGTAATTA TCCGTGGTCA TCAGAAACAC ATCACTGACA
26461 GACCATTTCT GTGTTGTCAG CCACTGGGTG CATTGGAACA GAAAGCTGAT TAATTGCGTT
26521 AATGCTGTAT CAGAAAAAAG GGCAATTTTC GTGTTCACAT AGGGAGAAAC CGACAACAAC

Fig.2.

26581	ATGGATAATT	CATTCACTGT	CAGATGATGA	ATGTCTGCCA	GCAGACGAAC	GCGATAAAGC
26641	AGAGACAGGT	TCTCGATGGA	ACACATAAAT	TCTGGATTTG	TTCCGCCATT	AGCCAGTTTC
26701	CATAATGTAT	ACAGTTCAGT	ATCATTCACT	CTGAAAGCAC	GTTTCATTAT	TCCCAAATAA
26761	AAATGGTTTT	TTGATTCACC	GGGGGTTAAA	TCCAGTTTGG	TATTATCAGC	AGAAAACTCT
26821	TGGCCATTTA	ATAGCGGTGT	ATTGAACAGC	ATTGTAAAAT	GACTGGGTTG	TTGTTTAGTG
26881	GAATATTGGC	TGATATCTGA	ATGACACAAT	ACCAGCGCAT	CGCTGACGCT	AATATTATAG
26941	TGCTGCATAT	AATATTGAAC	ATAAAACAGC	TTACCCAACA	CATTGCTGTC	AATGGTTAAG
27001	TCATCATAAA	TACTTTCTAT	TACTTGCCAG	ATATCTTCTG	GAGATATGCC	TGTGGCTTTA
27061	TACAAACGAA	TCGCTTTATT	CAGCTTTAAC	AGGAATATAT	CACCGGGAAC	TCCATCATTT
27121	TAAAGTGTGC	ATTGGCATTG	ATAGCATCCG	ACGGATTTGG	TTAACTCGCC	ATAAGCGGAG
27181	TGTTATACCG	TTGGTGATTT	GCTCTGTCGT	CAATTTAATG	GGAATACTGT	AATGGGTATT
27241	AGCAATGGGG	ACGAAATTTT	TATCTTGGTA	TATATATTCT	TTATCTCCAT	TCTGGAGACG
27301	AAAATCCAAG	TGGTCAGGTT	CTGTTTTTTT	TACACTGAAA	TTATATTTGT	ATTCATTTTC
27361	TTTGATTGGA	ATTAGCTCTG	CATAGTTTAA	ATGTGAATCG	TAGAAATCTT	TGCGGGTTCG
27421	CTTAATCAAT	CTTGCCGTTG	CCGTATCATT	CCCGTCATTG	ACCAATGTTA	TCAGTTGCTC
27481	ATTCTTATAC	TGTTGATTTG	TATTTTTCTT	ACCGAAGGAG	AGATTGACAA	ATAAACTGAG
27541	TTCATCATAA	GACAAATCGT	AGTAGCGAGC	CAAAGAAGCA	TAACTCTTAA	AAATCAGTAC
27601	ATCATCTGTA	CCGAAATTTT	TCTTCATCAG	TTCTGTTGAA	TTTTCCGGTG	TAATTTCTTC
27661	TACAAGGATT	TGATACAATT	CAGGCGATAT	ATCAGTCTTA	ATAGCCAGTA	GCGATGTTGG
27721	GTCCATTAAT	TCCGCTACGT	CTGTATTACG	GCTAAATGCG	GTGAGGTTTT	TATCTTGCAA
27781	TAAAATTGCC	TGACGGGCTG	ACTCATACGG	CAGATGATAG	GGTGTCATGC	CGGTTTGCCG
27841	GTAAGTGGAC	AACATTTTCA	TTACACCGTT	ATAGTCAGTT	TTCTCTAACG	TCTGAATATT
27901	ATGCAGCAGT	AATTCATTAG	ATAAGGATAA	TGTGGAAATT	TCTTCATCCA	TATTATTCTG
27961	TGTCAGTGCC	AGTGAAGCAA	TGTCGGGGCG	TCGTTTATTC	AGGTGATATT	GAGAATTGTC
28021	AGGATGAAAA	TCTTTCGCTT	CCCGATATAA	TTCTGTTAAA	TAAGCCGCTG	GTGAAAATAT
28081	GGAAGCAATT	GATCCCGGTT	TTACAAAACG	GTGGGCGCGG	CCATAAAACC	AACTGTTGTA
28141	ACTATTGTTT	AGGGTTGACG	GTGTAATATT	AAGGTTAGTG	ATATTAGCCA	GTTGTGGATT
28201	AGCACGGGAC	AAAATGCGCA	GTTCTTCAAG	TTTATTCTGT	TTTGATTCCT	GATGAGCCTG
28261	TTGATATAAA	AAGTCTGTTT	CTCGCCACGT	CAGAGTTCCA	CTTGTCCTAT	GACGAAATTC
28321	GCTGAAAGAC	ATAAACGAAA	TGTTTGTCAA	TAATAAAGTA	TCACCAGCCT	TTTTCTATTT
28381	ATCTTATCTA	ACAGTTCATT	AACTTTTATC	ATATAAATCC	TTAAGTTATT	GTCAATTTAA
28441	TGATTAATGG	TTTTTAGGTG	GAGATTATTA	TAATCTGATA	GGAATATTAT	GGTTAATTAA
28501	ATTGATACTG	ATTTATCGCT	CTATTCTTTC	AATAAAAAAT	AAAGAACTTC	CCTATAATAC
28561	ATGGATTTAA	ATAATGAATA	CCGTATGTTA	AAAATTAAAT	TTTAACAAAC	TTTCATGAAA
28621	AAATTCAACT	CAACAATTGT	TTAAATATTT	TTAATTGTGT	TTGTGCTGTT	TGAAAAATGA
28681	ATGACTAATA	TTTATCTATG	AAAGATTATT	TATTGAGGAT	GTCTTGCTTG	GTTTCAGGGG
28741	GCTACGTTGG	AGTCAGATAA	ATGTGTGCAA	AAAGAAATCC	TTAATAAAGT	TGCGTAATTA
28801	CAAAAGTTGG	TATATCGTGA	CAAGAGTGAT	AGTAATGTCA	CATAATTTAT	TGAATACCCG
28861	AACCTCGCAA	ATGCGGGGTT	TTTCTTCGCA	TAATCAAAGA	GAAAGCTATG	AAAAAAACAC
28921	TGATTACTCT	TATTCTCAGT	ACCCTTTCTT	TTGGTGCTTT	GGCACAGCAG	GGTGGCTTCG
28981	TTTCCCCGGA	CAGCACAGAC	TATACTCAGG	GTGGATTTAA	AGGTCCAACT	CCCAACCTGA
29041	CCAGCGTTGC	TCAAGCAAAA	TCTTTTCGTG	ATGATGCGTG	GGTTGTTCTG	GAAGGAAACA
29101	TTGTTAAACA	GGTTGGTCAC	GAACTCTATG	AATTCGCGGC	CGCATAATAC	GACTCACTAT
29161	AGGGATCGCT	TATTACGGAC	TTATCCGGAA	AGCTATCTGG	AACCCCTGTT	ACGCCTGAAT
29221	AAAACAGAAT	TCAGGGATAA	CAGTGGTTCT	GTTTATGTTG	ACATTGATGA	TAAGCGCTGG
29281	ATGGGTCTGA	CGGCCACTCC	AACTGACAAA	GTTCGTATCG	AAGGTGAAGT	GGACAAAGAC
29341	TGGAACAGTG	TTGAAATTGA	TGTCAAAACT	ATCCGCATAG	TGAAATAACT	CAAGCACTTT
29401	GAATATAGCC	CCGCACTCGC	GGGGTTTTTT	GCTTTCTGGG	AGTCGGAAGT	TTAACCGTAG
29461	TGACGAGGAT	CAAAACTAAG	TTAACGGCAG	TGGTCACTGA	TTTGGTGCAT	AAGTTATCAA
29521	AAGTTAAAAA	TCAAAACTTA	TTTTTTATTT	AATAGAGGAA	TGTCACCCTG	TAGGTGAATA
29581	ACGTTGACGG	ATGTAAATAT	ACAGTATTAT	AGTCCTTTGA	TATGTTATTA	AATTGAAAAA
29641	CCTTTAAACT	ATATTCGGGG	GAAATTATTA	TGTCAGATGT	TCGTAATATT	ATTAATGTTG
29701	ATAACAATTT	TGGTTGTGAA	TATAAAGCGG	ATTTATTTAA	ATAAGTTTTC	ATAATTGTGA
29761	TACACCCATT	TTTCTCATCC	CCGGTTTTTG	CTGTTGTAAG	GAAGCGGTTT	CCATGAAGAT
29821	TTTGACATGG	TTAAGCAACT	GCCACATAAA	TTGGCAGCAG	TGGTTTCGTG	TCACGGTTTC
29881	ATGCAAGGAT	TGCCATAGAC	GTTCAATTTT	ATTCAACCAC	GGGCAATAGG	TCGGTAAAAA
29941	GAGAAGATTA	AATTTGGGAT	TCTTTGCCAG	CCAAACCCTG	ACCTTCCGGC	TCTTATGAAT
30001	GCAATAGTTA	TCTAAAATTA	ACGTGATGGT	TTTGGCATTA	ACATATTGAT	TGTTAATTTC
30061	ATCTAACAAT	TTGATAAATA	AATCTGAGTT	CTTTCTCAAG	CTACCGACAT	AAGTGATTTC
30121	TTTCGTTTTC	GCGTTGAGGC	AATTGGCAAG	GTAGTGTTTT	TGGTTCTTTC	CGGGGGTAAC
30181	AACACGCTTT	TGTTGCCCTT	TGAAGCACCA	GTCTGCACCG	ATTTTCGGGT	TCAGGTTGAT
30241	GTCCACCTCA	TCCTCATAGA	AGACCGGGTG	TTTCTCTTGA	GGCATTGGAT	AACGTCTCGC
30301	TGATTTTTGC	CATTTTTTCA	TCATACTCAG	GGTCAGGCAA	TTTTACGGTT	GGTGCCGCCC
30361	TTCGCCAAAC	GATGCCCGTC	CGGCAAAAGT	AGCGATAGAG	GGTACTTTGA	GAGAGCGATG

Fig.2.

30421 TATTCAGTAG CTCATTGATT TTAAGTGTAA TAAGCTCAAG GCTCCATCGT GAACGGAGAT
30481 AGCCAAAATG TTGTGGCGAG TGCTGTAATA AGAAAGAAAT GACTGTGAAG AGCGGAGCTA
30541 AGTTCCAGAT GGCAGGCCTT CCCGCCGGGA GGCTTTTAAG TCCTTCCAAC CCGTATAATG
30601 TTAACCAATT TACCCAACGA TGAACGGAAG AACGTGAACA GTGAAGCGTT CTGGAAACGT
30661 GAGAAACCGT ACTCCCTTCA TGTAACATCA AGAGCGCGGT GAAGCGACGT GCATAGTCCT
30721 TATCCCGGGT TTTCTGGATA GCTTTTTTCA TCGGACGTCG TTCATTTCGG GGTATTGATG
30781 TTATGATTGG CATGACTCAG TCCATTTTGG GATTTGTTTT GATTTGGCGA TTAATCAGAT
30841 CGCGAAAATC GGACTGAGTT CCCTTCAAGT GATCTACTAT TTTGAAATCT TATTTAATCA
30901 GGAGTCAGCA AATGAGTTAT TCCCCATAAT ACCTGACCAT GTGGTTGTTT ATCCGGGAAA
30961 TGATTCATCT ACCGGTGGTA TGTGGATTCC TTGGTGCGAT AGTCAGAAAG ATATTGACTC
31021 TGGCCATTAT ATCAAAGTTA CTTTCAGTAA AAAGGACGCT GCTGATATTG TGAACTACAT
31081 GTTTCAACAT GGCAGTTATG TTTATTTTAC AGACAGTAGT AAACAATTTA GCAATAAGCA
31141 AATTATGTCT GGTGATTCAG CTAAAGGCAA AGGGGATTAT AAGCTTGAAA TTAAAACAAA
31201 CGGGAACCTT CCACTGATGG TATTGAATAA ATATTGATTC ATTATTATTT ATGGATAAGA
31261 AATTAAGTTT ATATTTCATC TGGTTTCTGC AATTAAGTTT TAAAAATTAA TTCTACTTTT
31321 TTTATGGTTT TATATTTAAT GCCAATCATA TTATTTTTCT TATAATAATT GATAGTTTAT
31381 TTATATAGTA AATAAATTCT GTTGGATGTG ATTATTATTG TGAGACGGTA ATAATTAACA
31441 TAACAGAAAA TTCATGGTTA GGAAATTCAA TCAACTTTTG TCCGGTTTCC TGACCATGAA
31501 GAGCTGTATT TACTGTAGAA CTCGCATTGA TACTGGATTG ATTAGCCGGA CGAGTGTTGG
31561 GTCAGCAGAT AATATGTTGT ATATTGGCTG TGGATTTTTC AGCGAGATGA TAGCTTTGGC
31621 AGTAAAGGCG ATTAATAACC GATAAAACAG AGAGACGGAT TGTGGCCAGG AAAGCAAAAA
31681 AGCCTCACCA TGACGCGTTA TTCAAACATT TTTTAACCCA ACCAGAAACC GCCCGGGAAT
31741 TTTTATCCCT TTATCTGCCG GAAGCGATCC GGTCAGTGTG TGATTTACCA CACTAAAACT
31801 GGAACCGGCA GCTTTGTGGA CAGGCAATTA CGTCAGTTGC ACAGTGATGT GCTGTATTCT
31861 GTCGAGACAA CCCACGGGGA CGGTTACATT TATTGCCTGA TTGAACACCA GTCCACGCCT
31921 GATCCGTTAA TGGCCTGGCG GCTGATGTAT TATTCGCTGT CAGCCATGGC TGCGCATCTG
31981 AAAAAAGGAC ATACTGAACT CCCTTTGGTC GTCCCCCTGC TGTTTTATCA TGGTGAGGTG
32041 AGGCCTTACC CTTACTCAAA TCGATGGCTG GATTGTTTTA CACTCTCTGA ACACGCGGCT
32101 CACCTGTATA ATCAGCCCCT GCCGTTGGTG GATATCAGTG CGCTCAGTGA TGAAGAGATC
32161 CTGACACATA AAAGCATTGC CTTGATGGAG CTGGTACAAA AACATATCCG TTGCCGGGAT
32221 ATGCTGGAGT GGGTTCCCCA ATTGGTGGCG TTGTTGAATG CCGGTTATAA TAGCGCCGAA
32281 CAGCGCCATG TTGTGTTAAG CTATATTTTA CTGAATGGAC ATACGCTGGA TCTCGCCCAG
32341 TTTGTCCATC AACTGACTGA ACAATCTCCG GAGCATGAAA CCATGTTGAT GACTATTGCA
32401 GAACAGCTTG AACAAAAAGG GCGTGAGCAA GGCCGGACAG AAGGCAGAAC AGAAGGCAGA
32461 GCTGAAGGAC GGGAAGAAGG CAAGCTGGAA ACGGCGCGCG CATTATTACG GCATGGTGTC
32521 AGTCTGGACA TCATTGTCAC CAGTACCGGC CTGAGCCGGG AGAAAATTGA AGCGTTAAAG
32581 CATTAAATGG ATACGCTTTT TCACAGCAGG ATATGGTGAC CCCTGTGAGG CCACCGGAAA
32641 ATTTTATTTA CTACGATTTA CGACGGGTTA CTTTAGGAAG CTGAATGAGA CGTCCTTTGT
32701 TATATAACGG TCCCATATCA ATCTTCTCTT TTCCGCGTAC AGGTAAGTAA CCCAAACCTT
32761 CGTGAGCAGC ATTTGCCAAC AGGCCATCAT CCTGATCGCC TGACCAAGAG AAGATCCCGC
32821 CCAATTTCAT TTTGGTTGCA TAAATTCCCT TATGCAGCAC AGTGCGGGGC GTATCCAGTG
32881 AAATCCAGTG ACCACCGTCA GCATTAAAGA GTGCGTCAGC GTCGGTTTCC GTGTCTGTCA
32941 CCAGTTCAAA CTGATTTTTC CCGCGTGCAA TTTCATATTC CGCATCGTAT TGGTTATTCA
33001 GCAGACAGAA GAATTCCGGA GCACCTTTTT CCATCGTGCC CAGTGGCTCT CCTGTTCTGT
33061 TATAGCGGCG CGTTGTCAGA TCAGCACCCA GACATGAACG TCCATAGTTA GCAAATCCGA
33121 GGTGAATTTT CTCCGGTTGT ACACCTTGTG ACAGTAAAAA GCGGATCGCC TCATCTGCCG
33181 AGTAATCCAT GTCCCGATCA GGATTGGGCG GAGGAGGGTT ATCGCCGTCA TATTCATATC
33241 TGGGGGGATA CAGGTTAGTA TGGTGACCGA TGTATTCTGC CCAACCGGTA CCAAAGAAGT
33301 CGTAGGTCAT CACAAAGATA TTGTCTAAAT AAGGTGCGAT TTCTTTGAAG CTGGACTTCT
33361 CCATTTTGGC AACGACGGCG CTACAGGCTA TCGTGATTTC TTTACGGGCC CGGGTTCCAA
33421 AGGCGATGTT CAGTGCTTCA CGCAGCTCTT TCACTAACAA AACATAGTTT GGGCCATCAT
33481 GTTCCGGGTC GAATTCATTA CCTTCTTCAC CTGTGGCGCC GGGGTATTCC CAGTCGATAT
33541 CCACCGCAGT AAACATGGGA AAACGCCGGG AAGAAGTCGA CGATGCTACT CACAAATGTA
33601 GCACGTTGCT CAGGATCTTT GGCCATCACA GAGAAATACC CTGACATACT CCAGCCGCCG
33661 ATACTGAATG CGAGTTCCAG CTTATGCCCT GCCTGTTTTG CTCGCGCTTT CAGATTACGC
33721 AATCCCCCCA GTAAACCGGA GGCTGCATCC TGATTGTAAT ATTGCAAGAA ATTCTTCGGG
33781 CTGGCATCAC GGCGCTGATC CGCGTCCAGA CCGACATTGC GTGTGGTGCC TAAATCACCA
33841 TAAGGATCAA CGGGTACAAT ATGGCCTAAT GTAATAGGGG CAATCTGGCC ACTGCTGGCT
33901 TCTGCTTGCC GGTTCCACCC GTCAACAACC TCATTAATCC GTTCGGATAA CTTGCCTTTG
33961 TCACCGTTGA CGGCCATAAA ACTGAAAATC AGGCGGTCGT AGGCGGTAGG CGGGATTTTT
34021 TCCAGATCAA AACCACGGCC GGGGGCATCG TCGCTGGTCA GCGCAGTGTT ATCCTGGGTT
34081 TCTGGCGACA AACGCGCATC ATACTGGCAC CAGTCAGTAA TATAGGCAGA GACTTTAGGC
34141 AGCGGTTCTG TATTTTCCGG ATCAACTTCA TATTCGTTGT ACAGGGACTT GGCAACACGT
34201 GCTGAAGAAT AACTCAAAGG AGTTCCGCTG CCGTCAGGTT TATATCCCAC CTTCTGATAG

Fig.2.

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34261 GTTTCTTCTG TGAGTGCATC ATATTGCAAT ACCTCGGTTT TTTCTCCCGG CGGTACATCA
34321 GGCGTATTGG GGTTACCGTG ATCGGCAATT TCTTCCGGTG TCGCCTCACG GACATATTGC
34381 CAGGCATTCT CATAAACCGG TAAATCAGGT GAAATATTGC GGTCGGGAAT ATGCCAGCGT
34441 TCAACCCAGC CGATGTTTTT AAAAACCGCG CTATCATAAA TGACATACCA GGTTTGACCA
34501 CCAGATTGAT TCTGCCAGGC AACCAGAGAT GCGCCTACTT CGCTGCTGGC GTCAGACATC
34561 GCTTTAATTG AAGGGTATCG ATAAACATTT TGAGACATAA TTTCACTTCC GGCCCCGTTA
34621 TATTCCGGGG CCGGCTCCTG ATATCAGTTA GAATTGTCTT GTTTTAATTG ATGTTTATTC
34681 AGACGGCTAC GAACCTGCTG GCTGAACTCA TTACTTCCGC CACTCACATC ACGCGCGGTA
34741 TAACGCAGAT GGAGGATAAT ATCGCTCAGC GACTCCAGCA GCTGATCCTG ATCGGAACCG
34801 AATTCCAACT TCCACTGTGA AATGGCGCCT GTCCCTTCAA AAGGCAGGAA AAGTTCATCA
34861 TCAAAATTGA GCCTGAACAT GCCGCTGTCT TCCATGGCCG TTGAAATCAC CACACCTTGA
34921 TTAGCCTGTA CGTTCAGCAA AACGTTTTCG GGTTTGGTGT ATTCCAAGGG GTTAAGCAAA
34981 TAATCGATAG TTTTTAAGTC AGCAGTACTG TAAAGCGTAT TGCTGAGTTG TACCAGTGAA
35041 GCCCGTACAT CTTCATAAGG CCCCAGCAAT GCGGGCAATG ACAGCGCTAC GGTTTTTATA
35101 CGCCGATCAG CGTGGGTCGG ATAATCGCGC AAGAACATTT CGGCGCTCAG TAAGAAAGTG
35161 AATGAACCCG TACTCTTGCC AATTTCCCAC TGTGATGATG TCAGTAATGA TTTTACCGAT
35221 ATGGTTTTTA TGATCTCCAG ACGTCTGGTG TTATGTTGCA AATACGCCTG ATCCATCCGT
35281 TGTAAGGCTA ATTTCAGATG TTCTCCGACC AGCAGCCCCT GATAAAGATC ATTCCAGAGA
35341 CCACTTTGGA CGAAATTCAT ATCATACTGA CCTGTTTCGT ACTGCCAGGA GGCTTCGGCC
35401 AGTAAACAGA GGGAATTAAC CGCATCATAG GCTTGCAGGT AAAGCCGGAG ATTTGGCTGA
35461 TCATCCACAT GTATAACGCA TCATTGGTAN ANTTGTTCNN NNNNNNNNNN NNNNNNNNNC
35521 CCGAAGCATA CCGCCAAGAC CATCCCCCCG ACGGCCAGAC CGAAAATATT GGGAACCATA
35581 TCCGCCACAG CGGCCGCAGT GGCGGCTGAC TGGGCAGCGA TCACACCTTC AGCCGCTCTT
35641 GATTGTAATG CGATAACTTC CTGCTCGGTG ATGGAGATGT TTTCATCATA GAGCGATTTA
35701 TAGTGTTGCT GGCGCTCCTG AGCGGCCCGT CGGCTGATGG TCAGTGCATC CAATGAAGCC
35761 TGTTGCATGT CAATCGCTTG CTGTTGCAGA TTGCGGGTAA AGCTGTACAG CCCCAGTTGC
35821 TGCTGCATAC GGAAGTGTTC AAAATCGGTA TTGTCTTTTT TCTCCAGCAA ACTCAGTAAC
35881 GTGCTGCCGT ACTGAATCAG CGTTTCTGCG GCCTCTTTTG CCCGGCTCAT GATCGGGGTG
35941 AAACGATAAT TCGGGATTGC CCGGCGTTTC ATGCCCGCCA TACGATTAGC CACAACACGC
36001 TGGTAACGCT GCCTGAGCAG ATCTTGCGGG CTGATGGGTT CATCGTATAA TCCGGCCGGA
36061 AACTCTTTAC CATCCAAGGT CAGGTTATGA CGTAAGTTAT ATAGACGCTG ATCCAACATT
36121 TGCCACAGTT TGAGATATTC CGTATCAACA GGTTTGACAA ATAAATCAGA CGGTGCGGCA
36181 GAGACGGATG TATCATATGT CACAGGCAGA AGTGGCACGT TGCTGACAGT AAGCATTAAC
36241 TCCTGTGCCC GTGCTTCACT GTTTTCATAC AGAGCCACAT CTTGCAGCGT ACGGGGTTGC
36301 CAGTTTGCCG CGAGCAGAAT ATCAGGGCTG GTACCCAGTA ACATATTGAC GGAGTCATAG
36361 ATCTGCTTGG CGACAGTACG TGCACTGGAT GTCAGCTTAC GGTATTCCAT GTCTCCCTGA
36421 TCTAACAGAT TCTTGACATA GAAACGGAAT ATTGCTTTCC GGTAGTGAAT GGGTTCACTG
36481 GCTGCAATGG CATCCGGATC GGTTGGTTCA ATTAACATCC GGTACACGGT GGGTGGAGGA
36541 TCAATAATTG GCCGTGAATT CCAGTAACGC GGTTTACCTT GGTTGCTGGC CTGAACAAGT
36601 TCATCTTCCA GCGGATTAAA AATATAGTGC AGCCATTCGG TGGCCTCTTT TAATCGTTGT
36661 TCTATATTCA GTCGCCACGC GACCAGAAAT GGCATATGGA AAAACAGTTC CCAGAAATAG
36721 ATCCCATTTG CGCCATTTAA ATCAATCGGC GTAGGGAATG AACCGGGTAT AGGCTGTTCG
36781 GTAATAAGCT GTGTATTCCA GCTCAGTACC TGCGGGATAC CCTGACTGGC AATGGCGATC
36841 AGTTTTTTTG CAAACAGTGT ATTAAGGCGA ATGTTTTGTG GCGCGTTATC AGTTTCATCT
36901 GCGGGGAAGG AAAGGAATTG CACCTGATCC TGTTCATTGA GTTTAATCAG TTCGCGAATA
36961 TGCATACCGA TTCTGAACTC TTGAGTACAG CTGGCACTTT CATTGCCAAC ACCACCTTTG
37021 GGCTTAAAGA GAAGTTCGGC TTTCAGGGTG ATTCGATTAT CCGACCCCAG CTTGATTGAT
37081 GGATAGGTTA AATCAAGAAC TTTTTCGCTC AGTACCAGTG GTTGTTCATC CAAGACAGTA
37141 TTATCGTGCA TCAGCCGGAA AGAACCGTTG TAATATTGAT GATCTTCTAT CGCACCAAAC
37201 TTAAAGTCAG ATTGAGCGAC AATCTCCAGT GTGTCATCAG TGCCATGAAC AAAATTGACA
37261 ATCAGTTTGA TACTGTCTTT GCCGAAATCA GGGTTCATTC CGGTTTGGAT TCTCCGGCAA
37321 TAGGAAAGCG TTCTTCCCGG GTTGCCGGAT AGAGCACCAT AGTACGGTAA TCGATAGGAT
37381 TGCCTTAAGG CATCCTTGTG TTCACGTGAG TAATACCAGA CCAGGTTGCC GACATATTTT
37441 CCTTTTCGTC CATCAGCATA TTGGTCATCC GGCAAATCAG TAATTTCTAC CAGCAGTGTA
37501 TCGCAGACAT AACCGAAGGC TTCGTCATAA TCATAATCCT TACCTTTCTT ATCTGTCCCC
37561 TGAAGACGGA CAAACGGAAC CAGAGCCAGA AACGGGTTAT GCGGGTCTTG CTGTATATCC
37621 ATCACAGCAA CCATCTGGGC CATCCGGTAT TGCAGATGTC TTCGCGCAGA ATGGTGGGTG
37681 TACTCCAGCT GCCATCATAT TTGGCATAAG CGATTTTGAT CCGGTCAGGA ACGGTGTGGG
37741 AGGAACCCAA TCACCCGCAC TAGGCTCAAC GTTTTGGTTA TGCAGTGATA ACGCAGTTGT
37801 ATCTTTAGTT TCAGACTGTT CTTCAACTTC CGTCCAGGCA ATATACAGGC GATTATTCAG
37861 GAAAATGGGG CGTATCAAAT TGGGGTCTAC GCTGCCCAAT GGCAGGTCAA TAGGTTTCCA
37921 CTCGCTCCAG GCATTGGGAG ATAACGCATC GGTATCAGGA TGGCGTATCG AAAGATTCAG
37981 TGAACGCCAG TAATATTGGT ATGGCTGTGT ACGGGTACGT CCGACAAAGA AGAACTTATC
38041 GCGTTTGATG TTAACACCAT CTTCATAACC TGCGATAACT TTCAGGTTAC TGACATCTTC
```

Fig.2.

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38101  AAAATTATTC AGATAACCGA GCACCGCTTG TTGTACAGAA TCTTCGGTAA TTTTTCCCTG
38161  ATTAAGGGCA CTTTCCAGTT GGAAGAAGAA TTCTGTTTTA TTCAGGCGTA ACAGGGGTTC
38221  CAGATAGCTT TCCGGATAAG TCCGTAATAA GCGATCCC
```
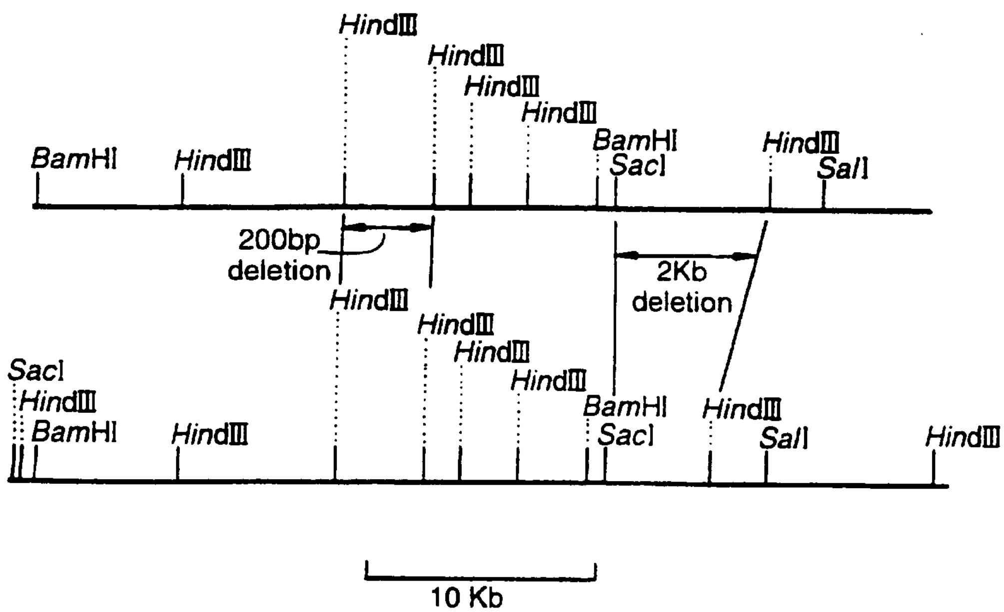

N=unspecified base

Fig.3.



INTERNATIONAL SEARCH REPORT

rnational Application No
PCT/GB 97/02284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/02 A01N63/00 C12N1/20 C07K14/24 //(A01N63/02, 63:02,63:00),(A01N63/00,63:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00647 A (COMMW SCIENT IND RES ORG ;SMIGIELSKI ADAM JOSEPH (AU); AKHURST RAY) 5 January 1995 cited in the application	1,5,11, 13, 18-21, 24-26, 29,30,32
Y	see page 1, line 3 - line 29; claims 10-13	3,4, 6-10,12, 14,27, 28,31

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1997	14/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muellners, W

INTERNATIONAL SEARCH REPORT

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PCT/GB 97/02284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 118, no. 1, 4 January 1993 Columbus, Ohio, US; abstract no. 3550, YAMANAKA, SATOSHI ET AL: "Biochemical and physiological characteristics of Xenorhabdus species, symbiotically associated with entomopathogenic nematodes including Steinernema kushidai and their pathogenicity against Spodoptera litura (Lepidoptera: Noctuidae)" XP002048914 see abstract & ARCH. MICROBIOL. (1992), 158(6), 387-93 CODEN: AMICCW;ISSN: 0302-8933, 1992, ---	3,6
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H.MATSUI ET AL. : "Nucleotide sequences of genes encoding 32 KDa and 70 kDa polypeptides in mba region of the virulence plasmid, pKDSC50, of Salmonella choleraesuis " NUCLEIC ACIDS RESEARCH , vol. 18, no. 8, 1990, pages 2181-2, XP002050055 see the whole document ---	21-25
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